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RELATIONS AND FUNCTIONS OF THE CLINICAL PATHOLOGIST IN THE HOSPITAL STAFF*

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The honor you have done me in asking me to speak to you tonight on the occasion of your Annual Banquet I should like to requite in a becoming manner.   I might perhaps do so by glorifying the clinical pathologist as he is but that would hardly be worthy of your intelligence even if it were in keeping with my own sense of scientific honesty.   For the clinical pathologist has not yet attained that rank, that degree of perfection of which he is capable; therefore I shall try to portray him as of the future, to picture him as some day he will be.   That method will inferentially bring out his present shortcomings.

The clinical pathologist is a product of specialization in medicine.   Most of us are of two minds regarding specialization—we see advantages and we see disadvantages in its development. That it is a phase of the evolutionary process in medicine cannot be doubted and as such is as inescapable and as inevitable as the machine in industry.   Its drawbacks to medicine are that it separates the profession into an ever increasing number of water-tight compartments.   The day of the universal scholar in medicine— of the Charcots, the Strümpells, the Jonathan Hutchinsons, is past and as unlikely to come back as that of the universal geniuses, the Michelangelos, the Da Vincis, the Goethes.   Prophecy however is always dangerous and perhaps I should not go so far as to say that such repetition of history is impossible, but scanning the world's horizon today fails to reveal the universal man either in the humanities or in the sciences, or for that matter, in statesmanship.

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We must therefore content ourselves with specialism and take advantage of its virtues, which are many. The greatest is that it provides the world with men of superior knowledge in a limited field. Such specialization has been of enormous value in medicine—witness the high development of neurosurgery, urology, bronchoscopy, ophthalmology, roentgenology. What a comfort and satisfaction it is to be able to appeal to men highly trained in these special branches for help and advice.

In a hospital with its complexity of cases any one of these and other specialties may on occasion be needed but there is one which is always needed by all departments, and that is the one which you gentlemen represent—that of the clinical pathologist, and by that token your dignity rises and your responsibilities increase.

Perhaps however Dr. Lynch is right in thinking that we may be asking too much of the clinical pathologist who has to be anatomist, histologist, immunologist, chemist and bacteriologist, but the very fact that so much is asked of him is in itself a compliment.

So much depends today upon the laboratory—Dr. Fishbein has said that a + or a − may determine life or death—that the position of the clinical pathologist in the hospital is well-nigh supreme. He is called upon to aid in the interpretation of symptoms, in the diagnosis of obscure cases and in the treatment of many diseases.

In order to discharge his multitudinous responsibilities and to make use of his new-born opportunities it is of the greatest importance for him to keep in touch with clinical medicine. The test-tube pathologist cannot fulfill the function that pertains to your position.

There are a number of ways in which contact with clinical medicine may be maintained:

1. By occasional visits to the wards to see interesting cases. The clinical pathologist should of his own initiative make such visits; at the same time the members of the staff should encourage him and welcome him on the wards.

2. By a discussion of the clinical aspects of cases coming to autopsy.

3. By conducting jointly with the other members of the staff clinical and clinicopathological conferences. By the former I mean conferences on living patients either in the wards or in small side rooms, conferences attended by interns, staff and students. Not only are such exercises of enormous pedagogic value for those who attend them but they bring the clinic and the laboratory together in an ideally helpful way.

4. By active participation in the work of an intramural medical society.

All these measures help to promote contact and coöperation between the clinic and the laboratory which it will be agreed is one of the greatest desiderata in medicine. It will be easier in the future when clinicians have a sounder and broader training in the fundamental branches.

On his recent visit to Philadelphia Prof. v. Gröer of the University of Lemberg told me that before engaging in pediatrics as a specialty, he spent five years in the fundamental branches of biochemistry, physics, physiology and immunology.

Under an ideal arrangement the clinical pathologist is a full-time attaché of the hospital. That status need not prevent his answering an occasional outside call in consultation or doing a limited amount of laboratory work for the medical profession. Such activities however ought not to interfere with his primary duties to the hospital and to the staff.

These duties I should now like to elaborate a little more fully.

First of all it is, I think, important that the clinical pathologist create enthusiasm among the medical personnel of the hospital for autopsies. Although the chiefs in the wards have a similar duty, the pathologist can do a great deal in this direction by making autopsies interesting. In my days as resident physician and afterwards as pathologist the autopsy room was always filled with interns who wanted to learn. I realize that morbid anatomy is no longer the drawing card it was a generation ago. For this waning interest, which I consider most regrettable, the pathologist is largely to blame.

I do not believe that the clinical pathologist can ever achieve a really high position unless in addition to being a chemist and immunologist, he is a good morbid anatomist.

It should be remembered that for nearly a hundred years the actual leaders in medicine have nearly always been pathologists in the anatomic sense—Virchow, Rokitansky, Orth, Prudden, Welch, Councilman, Aschoff, Hektoen, Flexner, Warthin. Such men are the court of last appeal in the diagnosis of tissue changes. No matter how far chemistry and immunology may go, a profound knowledge of morbid anatomy and histology will command the highest respect and will single out the man who possesses it.

Another duty of the clinical pathologist is to teach the interns the principal tests and laboratory procedures needed in the clinic. In order that this be done properly the pathologist must be interested in teaching. He must be able to select the essentials and must have the interns for an adequate length of time. Four months is, I take it, the minimum.

He should however not limit his teaching to the interns. The senior staff in most hospitals stands also in need of instruction. In every institution there are men who deride the laboratory, although they are usually the ones who make the most indiscriminate use of it. Such men must be tactfully educated to a knowledge of what the laboratory can do and what it cannot do, and that it is economically wasteful and scientifically irrational to have all manner of tests done routinely.

On the other hand the clinical pathologist must guard against overemphasizing the importance of the laboratory, else the interns will go out with such blind faith in laboratory methods that their confidence in the time-honored, never antiquated procedures of history taking and physical diagnosis is undermined. Only the man who knows something of clinical medicine can steer safely between Scylla and Charybdis.

The pathologist should stimulate the research spirit among the interns and should have no end of problems for assignment. If these problems can be different phases of the same major research, so much the better.

I was once in the laboratory of Professor Emil Fischer in Berlin. If I remember correctly there were 167 places in one enormous room, all occupied by men working on different problems in biochemistry under the unifying influence of the great master.

I shall never forget my attendance at a so-called colloquium at which various men presented the results of their work.

That the report from the clinical laboratory should be reliable and prompt is a sine qua non although not yet attained in all hospitals. But no matter how reliable the reports may be the clinical pathologist must not be offended if the clinician does not accept them in blind faith. Laboratory reports must be judged in connection with the clinical findings.

Only recently I had a case in point. We had obtained some fluid from the chest of a patient and had sent it to the laboratory. A dogmatic report came back "endothelioma of the pleura." The physical examination and clinical history pointed to long standing cardiac disease and not to tumor of the pleura. The autopsy confirmed the clinical diagnosis and revealed no pleural tumor.

If there are enough interns to organize an intramural medical society, the clinical pathologist should be a leader in the organization and should give to the meetings a scientific tone.

He is in the best position to create a true esprit de corps, not only among the interns but among the staff as a whole. Such a spirit soon makes itself felt beyond the walls of the hospital and becomes one of the chief determinants for medical students in choosing that hospital for their internship.

A pendant to the medical society of the hospital is the library. As his department will undoubtedly make the most use of it, the clinical pathologist has the deepest interest in its maintenance and expansion.

The clinical pathologist in a small hospital in a town or city not having a medical school and far away from a medical center has a splendid opportunity of making his hospital a school of postgraduate study for interns and staff.

Under his inspiration such a hospital may become a nucleus from which a scientific spirit irradiates the entire medical profession of the community. This has been well pointed out by Funk.

The growing employment of technicians in hospitals and by physicians in private practice makes it desirable that there should be schools for this modern female species. I see no objection to

such a school being attached to the Department of Clinical Pathology in a large hospital, provided that it is conducted properly and with due regard to the primary needs of the hospital itself.

And now I come to a matter that is not strictly scientific and yet I dare not neglect to speak of it, and that is emolument. The laborer is worthy of his hire. The clinical pathologist considering his responsibilities, his devotion to an intramural job that takes him out of the current of life, ought to be well compensated, although not too well. I say not too well for the same reason that makes me differ from President Hutchins of the University of Chicago, who not so long ago stated that a professor should receive at least $50,000 a year salary. A clinical pathologist or a *professor receiving such a magnificent wage would soon be* bothered by his investments and in a few years might be so rich that his wife would insist on his retirement from active duty.

There is one danger connected with the clinical pathologist's compensation, that is the danger of commercialism which the whole profession faces in this era of economic upheaval. The danger must be combated on the basis of the high ethical ideals that are the tradition and the pride of medicine.

I have sketched the ideal clinical pathologist as I conceive him. To achieve the state of distinction I have in mind he must overcome any inferiority complex; he must feel himself the equal of his colleagues on the clinical side and not merely a super-technician. Then he will have that flattering and responsible position for which the time seems to be ripe—namely the position of consultant-general.

PROPHYLACTIC VACCINATION AGAINST TULAREMIA

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Several writers have commented upon the high incidence of infection among laboratory workers engaged in research on tularemia, particularly among those who handle and perform autopsies on infected animals. Serious warnings have been given to those engaged in or about to engage in such work. That the import of these warnings is based on considerable experience is attested by the reports of Francis, Ledingham and Simpson. It has been stated that no one who has autopsied animals for four or five consecutive months has escaped infection. It has further been stated that no vaccine or serum has been proved to have any protective value but I have been unable to find any records of experiments bearing on this point.

Because of the seriousness of the situation for the laboratory worker and the general belief that prophylactic measures are useless I wish to report my own experience with prophylactic vaccination during a laboratory investigation of tularemia of eight months duration. During this period more than fifty animals, chiefly guinea-pigs, have been infected and autopsied. Cultures of *Pasteurella tularensis* (*Bacterium tularense*) have been made on various mediums and vaccines and suspensions have been made in many different ways. During this period the writer has had no illnesses. At the time of writing animal experiments are still being carried on and vaccination is being continued. It is regretted that this experience is limited to only one individual.

On October 20, 1930 a guinea-pig was infected with *P. tularensis* subcutaneously with pus from the finger lesion of a patient in the General Hospital two days before her death from tularemia. Since that time the strain has been kept alive on coagulated egg yolk and cystine-ascites-agar mediums with frequent passages

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through guinea-pigs. Suspensions of the bacteria have been
made by washing the growth from cystine-ascites-agar slants
with either 1.0 per cent formaldehyde or sterile salt solution.
Because of Wherry's previous experience that heat-killed suspen-
sions were very toxic, other methods were tried to obtain an
antigen which would be available for immunization, that is
one that could be given in adequate dosage without untoward
symptoms and yet be sufficiently antigenic to produce immunity.

Such a vaccine was finally made by treating a heavy suspension
of the bacteria with 1.0 per cent formaldehyde for twenty-four
hours, then with 3 per cent hydrogen peroxide for forty-eight

TABLE 1

The Relation of Agglutination Titer to Elapsed Time During the Period
of Continued Vaccination

SERUM DILUTIONS	NOVEMBER 14	JANUARY 8	FEBRUARY 2	APRIL 1	JUNE 1
1:640	0	0	0	0	0
1:320	0	0	0	0	+
1:160	0	0	0	+	+
1:80	0	0	0	+	+
1:40	0	0	+	+	+
1:20	0	+	+	+	+
1:10	0	+	+	+	+

0 = no agglutination; + = positive agglutination.

hours and finally with 1.0 per cent sodium rincinoleate for twenty-
four hours, washing the bacteria thoroughly between each chemi-
cal treatment, and finally resuspending in salt solution.

Beginning on November 14, 1930 with a minute amount of a
dilute suspension of this antigen, and with no agglutinins for
P. tularensis in my serum, I have vaccinated myself almost daily
with this, and later with various other and denser antigens,
gradually increasing the dose whenever that of the previous day
failed to give more than a slight local reaction, a simple erythema
not larger than a centimeter in diameter. Considerable care
was taken not to produce constitutional symptoms. However the
first eight subcutaneous injections produced a moderate amount

of general malaise, beginning about six hours after injection and lasting about four hours.

During the next three weeks only occasional general symptoms were noted, occurring only at times when the dosage was increased. After the first month no general symptoms occurred. At no time were any of the reactions very disturbing. The table shows the gradual rise of agglutinins in the serum as the vaccination was continued.

It is believed that the vaccination has afforded some protection against infection. The animal autopsies were all performed with ungloved hands and in ordinary clothes. The animals were immersed in a 1:1000 solution of mercuric chloride for ten minutes before they were tacked to the board. After completion of the examination, removal of organs, et cetra, all instruments, utensils and discarded remnants of organs were boiled. The cadaver was boiled, the board sponged with bichloride solution and the hands washed with soap and cold water. This is a reasonably careful technic but not more careful than that of other men who have become infected.

Early in May an accident caused several drops of a fresh heavy saline suspension of living organisms to fall on one wrist and run along the arm to the elbow. It was not possible to free the hands until after the liquid had dried when the arm was washed with soap and water followed by alcohol. No evidences of infection have appeared. It may be contended perhaps that it has been simply good luck, both in regard to the long period of performing autopsies and to the contamination of the skin, but certainly the weight of recorded human experience is against it. Without making any undue claims it has at least been demonstrated that the vaccine has produced a steadily increasing agglutination titer in the serum of the recipient.

Before the vaccination was begun intradermal tests were negative. During the first month of administration intradermal tests gave some erythema and itching, indicating probably that some degree of sensitivity had been acquired. Since that time several intradermal tests have been made, some with large amounts of antigen and all with wholly negative results. This is

distinctly contrary to my experience with fifteen patients who had tularemia, all of whom were extremely skin sensitive to minute amounts of antigen for at least seven months after infection.

The presumed immunity has not been put to the final test of infection with living organisms but it is thought that sufficient evidence has been adduced to indicate that probably a certain degree of it has been acquired. The method is as rational in theory as prophylactic typhoid inoculation and it is offered with the hope that others who intend to work with the disease will give it a trial. It is not practicable for the future rabbit hunter but I do think it will protect the laboratory workers.

SUMMARY

The experience of one individual with prophylactic vaccination against tularemia is presented and discussed.

A method for making an adequate, relatively non-toxic antigen is described.

It is believed that prophylactic vaccination will effectively protect man against laboratory infections of tularemia.

Note: Since this report was written I have begun to vaccinate another man who has been allowed to come into close and frequent contact with infected animals. So far he has remained well during a period of exposure of more than two months. A new vaccine has been prepared by a different method which renders it superior in every respect to the one described above. The maximum tolerance for it, as judged by local reactions, has not yet been reached although daily dosages of one half billion organisms are being given. This is fifty times the amount of antigen conveyed by the former vaccine. Small initial doses have been increased rapidly to the present amounts. There have been neither local nor systemic reactions. The antigenic property has not been impaired inasmuch as this man's serum agglutinated *P. tularensis* through a dilution of 1:80 after the first thirty-two injections. For this short injection period this is a considerably higher agglutinin titer than that induced with the other vaccine.

To each 2 cc. of concentrated *P. tularensis* suspension is added 2 cc. of a freshly prepared 30 per cent solution of sodium nitrite and 2 cc. of 30 per cent acetic acid. The mixture is allowed to stand at room temperature over night and is then washed thoroughly with sterile saline solution. The commercially available Mulford concentrate, containing 10 billion organisms per cubic centimeter, is detoxified equally as well as the fresh living bacteria and its use offers the further advantage of approximate standardization of the densities of suspensions that are made from it. This vaccine can be made safely anywhere. Also it permits prophylactic vaccination to become a practicable procedure. I might add that my own serum agglutination titer was 1:80 on October 14, nineteen weeks after the last date of administering this vaccine.

THE EXPERIMENTAL PRODUCTION OF AGRANULOCYTOSIS*

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The disease now known by the terms agranulocytosis, agranulocytic angina, mucositis necroticans agranulocytica, sepsis with granulocytopenia, malignant neutropenia, granulocytopenia, and agranulosis, is probably a distinct clinical entity that has arisen during the past ten years. Occasional cases, however, were observed before 1922, as, for example, the one reported by Brown[2] in 1902, and the infant, reported by Leale in 1910, who in manhood was again observed by Rutledge et al.[2] in 1930.

When Schultz first described this condition as a clinical entity and gave it a name, it was thought by many that it represented only a peculiar and very unusual reaction of the leukocyte picture to some type of infectious disease. It would seem strange, however, that such a reaction would develop within the past decade and have been previously unobserved in any appreciable numbers, especially since blood counts have been done routinely in many large institutions for nearly fifty years. If the condition had existed to any large extent prior to 1922, it would probably have been described long before, since the diagnosis is easily made, as the condition appears suddenly, runs a dramatic course and usually terminates fatally.

Agranulocytosis may be defined as a disease characterized by marked diminution in the number of circulating granulocytes, this in turn followed by no infection, localized infection, or generalized infection. Apparently it is a distinct clinical entity, with a clear cut pathological picture, a definite clinical course,

with signs, symptoms and a blood picture that is as characteristic as that occurring in pernicious anemia. In agranulocytosis there is a loss of myelocytic activity of the bone marrow with much evidence to indicate that it is primary, followed by a typical blood picture and a definite clinical course.

Much evidence is at hand to indicate that the primary changes are those of the bone marrow, as evidenced by the studies of Roberts and myself,[20] in which we demonstrated the disappearance of the granulocytes for four days prior to the clinical onset. Furthermore, much evidence from necropsy examinations indicates the essential pathological changes to be those of the bone marrow as described by Piette.[18] Bone marrow studies made during the life of the patients have been described by Buck[3] and others. Roberts and I have now studied the bone marrow removed during life from five patients and find an apparent cessation of activity of the myelocytic elements and in some instances the complete absence of those elements although the erythroblastic tissues were normal.

I believe that it can be reasonably assumed that agranulocytosis is primarily a disease of the myeloblastic tissues, followed by loss of resistance resulting in overwhelming infection. If this be true, the next question is, what agent is responsible for the cessation of myeloblastic activity.

There has been much speculation and divergence of opinion as to the cause of this disease. When Lovett[15] first reported the condition in this country in 1922, she found *Pseudomonas aeruginosa* (*Bacillus pyoceaneous*) in the oral lesions, whereupon she attempted to reproduce the granulopenia in laboratory animals with this organism. She succeeded in producing a mild granulopenia, but close study of her results indicates that the degree of granulopenia which she produced could often be observed in normal rabbits and guinea-pigs.

Pseudomonas aeruginosa which I recently isolated from the blood stream of a patient dying with agranulocytosis, failed to disturb the blood picture of rabbits and guinea-pigs. This organism has been frequently observed in ulcers in the mouth and in two instances in the blood stream, and therefore has been sus-

pected as a possible etiological agent, but in no instance, to my knowledge, has it been used successfully to produce agranulocytosis. This does not mean, however, that it should be ruled out as a possible causative factor.

Various other organisms have been suspected as being responsible for this disease, including all of those that have been isolated from the ulcers and blood stream of patients with the disease. In many instances, efforts have been made to produce granulopenia with these organisms, but apparently with little success. I have injected various organisms isolated from the blood stream of patients with the disease, and in each instance have failed to depress the leukocyte count. This, with the failure of others to reproduce the disease with bacteria, is further evidence that some other type of toxic agent depresses the bone marrow, resulting in an overwhelming infection by any or every organism that happens to reside in the gastro-intestinal tract.

There are some who believe that the bone marrow depression is caused by some type of spirillum that normally inhabits the mouth, or by a pathogenic spirillum that may be present in Vincent's angina. This belief has supportive evidence in the large number of patients that give a history of tooth extraction or oral treatment for Vincent's angina prior to the clinical onset. A review of the literature shows that nearly one half of these patients give such a history. This fact, however, can be variously interpreted. It is plausible to assume that these patients have mouth infections because of a lowered cellular resistance of a chronic granulopenia, or because of an unrecognized attack of acute granulopenia. Roberts and I[19] have stressed the occurrence of this condition, which is characterized by weakness, easy fatigue, and tendency to infection, especially in the oral cavity, where infection would most likely occur. It is my opinion that the high incidence of oral infection is a result and not the cause of chronic or acute granulopenia. In my last two necropsy studies of this disease, I examined bone marrow from the humerus, tibia, femur, sternum and ribs by dark field methods and was unable to find any organisms that would not stain with aniline dyes. I have also made dark field studies of five cases on the bone marrow removed during life, with similar results.

Dead bacteria and bacterial toxins also have to be considered as capable of depressing the leukocyte count, as evidenced by Bromberg and Murphy's[1] report of agranulocytosis following prophylactic typhoid inoculations, though it is questionable as to whether or not this was a mere coincidence. I[14] have reported a similar case in which a woman failed to recover from the final dose of typhoid vaccine inoculation, since she remained in bed with daily fever and extreme weakness for thirty days, culminating in three attacks of agranulocytosis.

It may be possible that the bone marrow depression is caused by a hidden or latent infection over a long period of time, so that when the resistance falls to a low ebb, acute infection follows, the myelocytic tissues being unable to meet the emergency. Adding support to this view is the number of cases that follow influenza after a variable period of time. Since the infecting agent of influenza tends to produce a leukopenia, it is quite possible that the residuals of the infection produce a slow and gradual damage to the bone marrow from which it is unable to recover. The low leukocyte count and extreme weakness following attacks of this disease tend to support this view.

In this modern trend of exposure of the human skin to rays of various types, as ultra-violet light, infra-red, sunlight, and roentgen, the possible effect of these substances should be considered. Pappenheim[17] caused leukocytes to disappear from the blood of rabbits by a single injection of thorium-X. Radiologists have long been aware of the leukocyte depressing factors in excessive dosage of roentgen-radiation.

Certain chemicals have long been known to be effective in the production of leukopenia. Arsenical preparations have been reported as being capable of producing this effect. Dodd and Wilkinson[6] have shown that arsenicals will depress the leukocyte count, though not to the point of agranulocytosis. A number of cases of agranulocytosis have been reported as following arsphenamine therapy. Then as a strange paradox, others advise the use of arsphenamine in the treatment of the condition. Talley and Griffith[23] report one of the few cases in negroes as following arsphenamine therapy. Arsenical preparations should be con-

sidered as possible etiological factors until definitely ruled out. Dr. Francis Carter Wood[31] has called my attention to the fact that he has found large amounts of arsenic on apples sold on the streets of New York, this arsenic having its origin in the wide use of insecticide sprays in orchards.

Benzene, however, is the only chemical that has been shown for many years to be capable of producing a marked depression of the leukocyte count to the point of complete agranulocytosis. Therefore it is the outstanding agent that should be considered as causative of agranulocytosis.

The most complete review of benzene poisoning has recently been published by Alice Hamilton.[10] Therefore a general discussion of the substance is unnecessary in this paper.

One of the first observations of the toxic effect of benzene on the myeloblastic tissues is that of Selling,[22] who observed three young girls who had been working in a rubber factory where they had been exposed to benzene fumes and then entered Johns Hopkins Hospital with agranulocytosis complicated by purpura and hemorrhages. Two of these patients died. The bone marrow was hypoplastic. The interesting features of these cases was the early chronicity of the symptoms, followed by sudden loss of neutrophiles and rapid death. This, in spite of the fact that one of them had long before quit her work, illustrating the cumulative and delayed effect of the chemical on the hematopoietic tissues. Selling[23] followed this observation by the injection of benzene subcutaneously in rabbits and succeeded in producing a leukopenia down to 200 cells per cu. mm. He concluded that benzene destroyed white cells of the circulating blood and parenchymal cells of the hematopoietic system, but that the circulating erythrocytes were little affected.

Winternitz and Hirschfelder[30] showed that a pneumonic exudate in benzene treated rabbits contained erythrocytes, fibrin, and endothelial cells but not neutrophiles. Kline and Winternitz[12] demonstrated that the loss of resistance to infection with tubercle bacilli was diminished. Camp and Baumgartner[4] were unable to demonstrate neutrophiles in benzene treated rabbits upon the application of croton oil to the ear, and Hektoen[11]

showed that resistance of benzene treated animals was decreased by loss of neutrophiles, reduction in antibodies and reduction in phagocytic activity of leukocytes present. Simonds and Jones[24] also showed that the immune bodies were much decreased in benzene treated rabbits. The most recent and extensive work on the action of benzene has been that of Weiskotten and his associates[26, 27, 28, 29], in which they showed that benzene produced an aplasia of the myelocytic tissues, a marked granulopenia, and that the life span of the rabbit neutrophile was about four days. They also showed that following the injection of benzene, there was a primary fall of the leukocytes, followed by a rise to normal, then a secondary fall, during which the animal usually died, the two falls being designated as the prophase and the deuterophase. Their injections were made subcutaneously, using 1.0 cc. of equal parts of benzene and olive oil per kilogram of body weight. They demonstrated that the urinary phenols markedly increased during and shortly after benzene administration. It seems, then, that the ability of benzene to produce a fatal outcome, due to a reduction of leukocytes, has been well demonstrated in both man and laboratory animals.

How does acute benzene poisoning differ from agranulocytosis? It has been generally accepted that benzene poisoning is accompanied by toxic effects on all elements of the bone marrow, resulting in decrease of erythrocytes, hypoplastic erythroblastic tissues, marked granulopenia, diminution in platelets, purpura, hemorrhages, and in some instances methemoglobinemia. McCord[16] discussed this differentiation, and Dameshek[5] accepted this view. Duke[7] stated that benzene markedly affects the platelets. However, it is equally true that the chief action of benzene is on the myelocytic tissues, and it is also probable that it can be administered in such dosage to produce marked granulopenia with no apparent effects on either the erythrocytes or the platelets, as shown by experiments described in this paper.

In a consideration of the etiological agents causing agranulocytosis, one must recall that the disease has been observed for ten years, that it occurs chiefly in middle aged women, and in a ratio of four women to one man. There is no age limitation as

it has been observed in infants, and a case reported by Roberts and myself[20] was in a woman of seventy-two years. The disease is not highly infectious and it occurs sporadically. It seems to have no seasonal variation, but since it appears to follow influenza in some cases, it may be more prevalent in the winter. The disease does seem to have geographical limitations, since about 80 per cent of the reports are found in the German and American literature. It is comparatively infrequent in France and England, rare in Italy, and extremely rare in the Orient.

This distribution could be correlated to the usage of organic chemical compounds. The development of chemistry has been rapid within the last ten years, and in Germany and the United States is found the widest usage of organic chemical compounds and aniline dye products.

It has been reported in individuals having various occupations and in many classes of people, school children, housewives, business and professional men and women, laborers, scrubwomen, physicians, nurses, farmers, prostitutes, and ladies and gentlemen of leisure; it seems peculiarly prevalent in the last named class, and it is practically confined to the white race.

The observations that the disease occurs largely in Germany and the United States, that it occurs in middle aged white women, for the most part in women of leisure or women living under good economic conditions, that it is seldom seen in a negro, that most of the patients have a history of previous medical care or treatment with various drugs, that the coal tar series of drugs has its widest range in those parts of the world in which the disease is most frequent, that these drugs and chemicals contain the altered or modified benzene ring, and that benzene is the one outstanding leukocyte depressant, lead me to believe that this substance or its products must be seriously considered as being the cause of this condition.

It is well known among industrial workers that negroes can work with benzene, aniline, dinitrobenzene, et cetera, with less danger of systemic poisoning than white men. Alice Hamilton[9] states, "Women are certainly far more susceptible to benzene poisoning than men."

In the production of leukopenia with benzene, Selling[23] and Weiskotten used equal parts of benzene and olive oil, giving daily injections of 1.0 cc. per kilogram of body weight. The olive oil was used, presumably to delay absorption, and the injections were subcutaneous. This involves a consideration of the mechanism whereby benzene produces its effect on the hematopoietic tissues, and the chief question to be answered was whether or not benzene or one of its oxidation products itself was responsible for the bone marrow damage.

Folin and Denis[8] state that "the oxidation of benzene to phenols and to dioxybenzenes is from a biochemical standpoint very interesting as a definite illustration of the vigorous oxidations which can and do occur in the animal body." They further state that among the various oxidation products the following are the most important: phenol, paracresol, orthocresol, pyrocatechin, hydroquinone, paraoxybenzoic acid and muconic acid.

Their work was based on the constant finding of these so-called urinary phenols during benzene administration. Therefore, it might be assumed that if benzene is injected subcutaneously in the animal tissues, it may undergo oxidation at the site of inoculation, and the oxidation products instead of the benzene be responsible for the toxic effects on the bone marrow. An effort was then made to determine the effect of benzene on the bone marrow by injection of pure benzene into the blood stream.

Experiment I: Three rabbits were given intravenous injections of 1.0 cc., 0.5 cc., and 0.25 cc. of benzene. Each rabbit died immediately.

Experiment II: Three rabbits were given intravenous injections of 0.18 cc., 0.12 cc., and 0.06 cc. of benzene. Each rabbit died immediately after injection.

From these simple experiments it would seem that benzene, when free in the blood stream, is extremely toxic for rabbits, and therefore, it seems unlikely that when the substance is given subcutaneously, it is taken up by the blood stream as such, but more likely it is oxidized to a less toxic product which probably acts upon the myelocytic tissues.

In view of this, it was then decided to determine the effect of the various oxidation products of benzene on the leukocyte count.

EXPERIMENTS

Rabbit 36: Given daily subcutaneous injections of 33 per cent phenol in olive oil for twenty days. It produced no effect on the leukocyte count.

Rabbit 34: Given subcutaneous injections of 1.0 cc., of a 33 per cent solution of tricresol, followed by eight intravenous injections of the same drug. It resulted in no depression of the leukocyte count.

Rabbit 47: Given daily intravenous injections of 0.5 cc. of saturated aqueous solution of orthocresol for five days, followed by the oral administration of 3 grains for four days, producing no effect on the leukocyte count.

Rabbit 48: Given daily intravenous injections of 0.5 cc. of saturated aqueous solution of metacresol for four days, followed by oral administration of 5 grains for one dose. No effect on the leukocyte count.

Rabbit 49: Given daily intravenous doses of 0.5 cc. of saturated aqueous solution of paracresol for eight doses, followed by oral administration of 5 grains for five days. No effect on the leukocyte count.

Rabbit 31: Given a 33 per cent aqueous solution of resorcinol (benzene oxidation product). One cc. subcutaneously for twenty days, five doses intravenously, failed to produce a depression of the leukocyte count.

Rabbit 43: Given daily intravenous doses of 0.5 cc. of a 33 per cent aqueous solution of resorcinol for eight days, followed by the oral administration daily of 5 grains for eight days, producing no effect on the leukocyte count.

· Rabbit 32: Given daily subcutaneous injection of a 33 per cent aqueous solution of hydroquinone (0.5 cc.) without effect on the leukocyte count. This was done for twenty-two days, then followed by eight daily intravenous injections, producing no effect on the leukocyte count.

Rabbit 46: Given daily intravenous doses of 0.5 cc. of a saturated aqueous solution of hydroquinone for five days. This was followed by a depression of the leukocyte count to 2,850. The administration of 5 grains daily by mouth for eight days failed to depress the leukocyte count.

Rabbit 33: Given daily subcutaneous injections of a 33 per cent aqueous solution of pyrocatechin for twenty days, followed by the same dose intravenously for eight days. It produced no effect on the leukocyte count.

Rabbit 44: Given daily intravenous injections of 0.5 cc. of 33 per cent aqueous solution of pyrocatechin for six days, followed by seven daily doses of 5 grains by mouth, producing no effect on the leukocyte count.

Rabbit 38: Given daily subcutaneous injections of 1.0 cc. of a saturated solution in 10 per cent alcohol of ortho-oxybenzoic acid (salicycle acid). It seemed to produce a depression of the leukocyte count, at one time reaching 3,300 with 92 per cent lymphocytes. Continuation with intravenous injections failed to affect the count.

Rabbit 41: Given daily intravenous doses of 10 per cent solution of ortho-oxybenzoic acid in 50 per cent alcohol (0.5 cc.) for five doses, this being followed by 5 grains by mouth for eight days. No effect on the leukocyte count.

Rabbit 39: Given daily subcutaneous injections of 10 per cent solution of para oxybenzoic acid in 50 per cent alcohol (1 cc.), followed by intravenous injections. It produced no effect on the leukocyte count, with the exception of mild leukopenia.

Rabbit 45: Given daily intravenous doses of 0.5 cc. of 10 per cent solution in 50 per cent alcohol of para oxybenzoic acid for five days, followed by 5 grains of the drug daily by mouth, producing no effect on the leukocyte count.

Rabbit 42: Given daily intravenous doses of 0.5 cc. of a 10 per cent solution of meta oxybenzoic acid in 50 per cent for six days, followed by 5 grains daily by mouth for fourteen days, producing no effect on the leukocyte count.

Rabbit 40: Given daily subcutaneous injections for twelve days of 1.0 cc. of a 10 per cent solution of meta oxybenzoic acid in 50 per cent alcohol, followed by intravenous injections, for five days. It failed to produce a depression of the leukocyte count.

Rabbit 37: Given daily injections of 1.0 cc. of 50 per cent alcohol subcutaneously, followed by six intravenous doses. No effect on the leukocyte count. This was given as a control, since many of the solutions used were alcoholic.

A summary of the results of the foregoing experiments indicates that intravenous administration of hydroquinone is capable of producing a moderate neutropenia. Also, subcutaneous administration or ortho-oxybenzoic acid, produces moderate neutropenia. Further work remains to be done with these two substances. Intravenous and oral administration of ortho-oxybenzoic acid, and subcutaneous injections of hydroquinone fail to produce this effect. Therefore, in a consideration of the depressing effects of chemicals on the blood forming tissues, the method of administration is very important.

The action of benzene on the myelocytic tissues

The chief object of the following experiments was to observe the action of benzene on the hematopoietic tissues, when given by subcutaneous, intraperitoneal, and skin application methods. Also to determine whether or not agranulocytosis could be produced without affecting the erythrocyte or platelet picture.

Rabbit 2: Female (Table 1). Was given 1 cc. of equal parts of benzene and olive oil subcutaneously daily for nine days. Erythrocyte counts, platelet counts, and studies of the erythrocyte picture, showed no disturbance of these elements. The rabbit died on the fourteenth day with 800 lymphocytes per cu. mm., no granular cells, a normal red cell and platelet count. On the eighth

TABLE 1

DATE 1931	LEUKO-CYTES	ERYTHRO-CYTES	NEUTROPHILS			MYELO-CYTES	LYMPHO-CYTES	EOSINO-PHILS	BASO-PHILS
			Seg-mented	Juve-nile	Banded				
3–18	7,600								
3–19	5,200								
3–20	10,100								
3–23	4,100								
3–24	2,050	4,770,000	2	12	20	4	61		
3–25	1,250	5,020,000				12	85		3
3–26	1,400	5,060,000	1	5		12	75	5	2
3–27	3,850	5,770,000		4		15	61		
3–28	600	4,140,000	4	1	32		63		
3–30	1,150	4,790,000					100		

TABLE 2

DATE 1931	LEUKO-CYTES	ERYTHRO-CYTES	NEUTROPHILS			MYELO-CYTES	LYMPHO-CYTES	MONONU-CLEARS	BASO-PHILS
			Seg-mented	Juve-nile	Banded				
3–27	8,000								
3–28	2,750		27		3		70		
3–30	3,150		30		2		68		
3–31	2,800		6				94		
4–1	3,000			5	1	4	88	2	
4–2	4,850								
4–3	5,500		15	10	6	6	60		3
4–4	3,800		32		8		60		
4–6	2,750				2	2	93	2	1
4–7	1,900			4		8	83	5	
4–8	10,250			10		28	59	2	1
4–9	1,300					5	95		
4–10	1,350			6		2	92		
4–11	4,600	4,300,000				24	76		
4–13	5,600	4,450,000	16	32		2	46	4	
4–14	7,200		8	8		20	52		
4–15	19,000	4,600,000							
4–16	13,450	4,950,000							
4–18	2,850	5,120,000							
4–20	1,400	5,000,000				16	84		
4–21	1,850					18	82		
4–22	1,100	4,700,000					100		

day the mucous membranes of the mouth were traumatized, injected and sprayed with an eighteen hour culture of diphtheroid bacilli isolated from the blood stream of a patient with agranulocytosis. At the time of death numerous oral ulcers were present. The condition was identical with agranulocytosis as seen in the human being. At autopsy, blood cultures were positive for a diphtheroid bacillus. Areas of congestion were present along the gastro-intestinal tract. Visceral organs showed cloudy swelling. Bone marrow showed an absence of granular cells. Animal died 3–31–31.

TABLE 3

DATE 1931	LEUCO-CYTES	ERYTHRO-CYTES	NEUTROPHILS			MYELO-CYTES	LYMPHO-CYTES	MONONU-CLEARS	BASO-PHILS
			Seg-mented	Juve-nile	Banded				
3–18	5,300								
3–19	10,100								
3–20	6,450								
3–23	7,850								
3–24	1,800	4,400,000	12		12		74		2
3–25	1,250	4,320,000							
3–26	1,350	4,380,000		12		22	61	1	
3–27	4,950	4,400,000					5		
3–28	3,900	4,060,000	32		30		38		
3–30	1,450	4,450,000	4				96		
3–31	1,300	5,080,000					100		
4–1	1,300						100		
4–2	1,150	3,270,000					100		
4–3	3,200					24	76		
4–4	1,850	3,170,000		10		35	55		
4–6	5,150	4,100,000		10		64	26		
4–7	5,100	4,530,000				36	61	3	
4–8	5,000	4,120,000		56		42		2	
4–9	3,600	4,500,000		12		74	14		
4–11	1,800	4,000,000				4	96		

Rabbit 3: Female (Table 2). Given 2.0 cc. of equal parts of benzene and olive oil daily, subcutaneously, for twenty days. Note the three falls in the leukocyte count, the animal dying with a leukocyte count of 1100, 4,700,000 erythrocytes and platelets normal. No hemorrhages were noted either before or after death. Autopsy showed aplasia of the granular cell elements, generalized infection, bilateral pneumonia with pneumococcus from blood stream. The picture was that of clinical agranulocytosis. Animal died on 4–22–31.

Rabbit 4: Female (Table 3). Given daily subcutaneous injections of 1.5 cc. of equal parts of benzene and olive oil daily for twenty days. The picture was

TABLE 4

DATE 1931	LEUKOCYTES	ERYTHROCYTES	NEUTROPHILS			MYELOCYTES	LYMPHOCYTES	MONONUCLEARS	EOSINOPHILS	BASOPHIL	DOSAGE
			Segmented	Juvenile	Banded						
											cc.
1–22	10,100										0.5
1–24											0.5
1–27	13,500										0.5
1–29	4,450										0.5
1–31	11,500										0.5
2–2											0.5
2–4											0.5
2–6											0.5
2–9											0.5
2–11											0.5
2–13											0.5
2–16											0.5
2–18											0.5
2–20											0.5
2–21	5,550										0.5
2–24	2,900		10	2	5		70	3			0.5
2–25											0.5
2–26	4,450		1		4		76	1		18	—
2–27			9	3	2	3	60	5		8	0.5
2–28	6,200		33	4	4	3	54	7		7	—
3–2			23		2		58	6		11	0.5
3–3	6,200		25	1	12		50		11	1	1.0
3–4			20	1	2	1	39	2	30	5	1.0
3–5	7,500		29	1	1		56	3		10	1.0
3–6			30		8		53	4		5	1.0
3–7	4,500		20	2	4	2	69	3			1.0
3–9	2,850		25		8		58	4	2	3	1.0
3–11	5,650		8				86	6			1.0
3–12	3,200	4,320,000	19		4		76	1			1.0
3–13	4,450		18		6		73			3	1.0
3–14	2,800		24		4		56		12	4	—
3–17	1,700					4	96				—
3–18	2,700					3	97				1.0
3–19	1,250		6				94				1.0
3–20	2,900*		2	2	8		86				1.0
3–23	6,750†		1	33		16	42				1.5
3–25	7,350			33	6		61				1.5

* Two myeloblasts.

† Seven myeloblasts.

TABLE 4—*Concluded*

| DATE 1931 | LEUKOCYTES | ERYTHROCYTES | NEUTROPHILS | | | MYELOCYTES | LYMPHOCYTES | MONONUCLEARS | EOSINOPHILS | BASOPHILS | DOSAGE |
			Segmented	Juvenile	Banded						
											cc.
3–26	7,050		15	17	11	2	55				1.5
3–27	12,250		5	39	10	15	31				1.5
3–28	2,300		29	13			51			7	1.5
3–30	2,000			2	14		83	1			1.5
3–31	2,000		4	2	5		89				1.5
4–1	1,400			2	6	2	90				1.5
4–2	800			4	12	2	82				1.5
4–3	1,150	2,800,000				8	92				1.5
4–4	500	2,340,000				5	95				1.5

essentially the same as that of Rabbit 3. Autopsy showed generalized infection with pneumococci, bronchopneumonia, and aplasia of the myeloid system. Animal died on 4–12–31.

Rabbit 5: Female (Table 4) Given subcutaneous injections of 0.5 cc. of equal parts of benzene and olive oil every second day, thus resulting in a chronic granulopenia for thirty-five days. The dose was then increased to 1.0 cc. daily, this reducing the leukocyte count to 1,200 after fifteen days (96 per cent lymphocytes), the dose then increased to 1.5 cc. for twelve days, the animal dying on 4–6–31 with a count of 500 per cu. mm., 100 per cent lymphocytes, 4,340,000 erythrocytes, platelets normal. Autopsy showed aplasia of the myelocytic elements of the marrow, generalized blood stream infection with staphylococci.

Rabbit 6: Female (Table 5). Given daily applications of benzene to the shaved abdomen for thirteen days, resulting in severe inflammatory reaction of that area, and a leukocytosis. The leukocytosis and inflammation persisted for ten days. Beginning at this time the animal was given subcutaneous injections of 0.5 cc. of benzene daily (no olive oil). After twenty-nine days, death occurred on 3–23–31 with 900 lymphocytes per cm., no neutrophiles, 4,430,000 erythrocytes. Autopsy findings similar to Rabbit 5 with pneumococci in blood stream. Apparently the presence of a preëxisting leukocytosis did not prevent the development of agranulocytosis. Olive oil did not seem to be necessary for use with the benzene.

Rabbit 7: Female (Table 6). Given daily intraperitoneal injections of 1.0 cc. of benzene in olive oil for sixteen days Animal died on seventeenth day (4–10–31) with 1,450 leukocytes, 4,200,000 erythrocytes, platelets normal. Neutrophiles 12, lymphocytes 88 per cent.

Rabbit 8: Female. Given daily inhalations of benzene for ten minutes for

seventy days. It produced no effect on the leukocyte count, though the lymphocytic percentages were rather high. Ten minutes was probably an insufficient inhalation period.

TABLE 5

DATE 1931	LEUKOCYTES	ERYTHROCYTES	NEUTROPHILS			MYELOCYTES	LYMPHOCYTES	MONONUCLEARS	BASOPHILS	DOSAGE
			Segmented	Juvenile	Banded					
1–22	25,050									On skin
1–27	33,200									On skin
1–31	14,650									On skin
2–3	27,300									On skin
2–5	19,800									On skin
2–7	15,400									—
2–10	15,450									—
2–12	21,000									—
2–14	15,000									—
										cc.
2–17	18,100									0.5
2–19	7,950									0.5
2–21	5,900									0.5
2–24	8,100									0.5
2–26	8,250									0.5
2–28	6,600									0.5
3–3	4,450									0.5
3–5	4,450									0.5
3–7	3,300									0.5
3–9	2,250		12		9		77		2	0.5
3–11	3,350		6		6		82	6		0.5
3–12	3,000	4,470,000	16		8		72	1	3	0.5
3–13	2,950		44		18		34		4	0.5
3–14	1,350						100			0.5
3–17	2,500		31	4	18		45	1	1	—
3–18	1,200		3	24	2	6	63	1	1	—
3–19	2,500			15		4	81			0.5
3–20	1,500				1		99			1.0
3–23	900	4,430,000					100			—

Effect of the aniline dyes on the leukocyte count

Because of the wide use of aniline and aniline derivatives in recent years, and because of the possibility that this class of substances may be instrumental in depressing the hematopoietic

tissues, four rabbits were injected with the following substances: aniline, para red, beta-naphthol, paranitraniline.

Rabbit 11: The abdomen was shaved and the shaved area painted daily with aniline for thirteen days, resulting in no disturbance of the leukocyte count. The animal was then given 0.5 cc. of aniline daily by the subcutaneous method for twenty-seven days, which also failed to affect the leukocyte count.

Rabbit 12: The same procedure and dosage, using a saturated alcoholic solution of beta-naphthol, was employed in this rabbit as in Rabbit 11. The leukocyte count was not affected.

TABLE 6

DATE 1931	LEUKO-CYTES	ERYTHRO-CYTES	NEUTROPHILS			MYELO-CYTES	LYMPHO-CYTES	MONONU-CLEARS
			Seg-mented	Juve-nile	Banded			
3-19	8,000							
3-20	11,500							
3-23	12,000							
3-24	10,600		45	1	12	10	32	
3-26	10,950							
3-27	8,000							
3-28	4,200							
3-30	1,900		38		8		54	
3-31	5,800		45		12	2	34	7
4-1	12,700		53	2	15		30	
4-2	13,150		32		5		61	2
4-3	8,200							
4-4	5,650							
4-6	4,950							
4-7	1,550							
4-8	850			32		4	62	
4-9	2,050	4,340,000	44				55	1
4-10	1,450	4,200,000		12			88	

Rabbit 13: The same procedure and dosage, using a saturated alcoholic solution of paranitraniline, was employed in this experiment, as in Rabbit 11. No effect on the leukocyte count.

Rabbit 14: This animal was treated the same way as Rabbit 11 using a saturated solution of para red dye. No effect on the leukocyte count.

A thorough consideration of the nine cases of agranulocytosis that I have studied reveals the fact that eight of the patients had been taking drugs of the coal tar derivatives prior to the clinical onset. This had occurred so consistently and inasmuch as this

class of drugs contains various modifications of the benzene ring, and in view of their widespread use, it was decided to determine whether or not certain drugs of that class produced any effect on the leukocyte count. The drugs used were phenacatine, amidopyrine, peralga, and dial, four proprietary preparations that had been administered in large quantities to four patients later developing agranulocytosis.

Rabbit 15: Given from 2 to 4 grains of phenacatine daily by oral administration for forty-five days, producing no effect on the leukocyte count.

Rabbit 16: Oral administration of 5 to 10 grains of amidopyrine daily for forty-five days. No depression of the leukocyte count; on the contrary, a persistent leukocytosis was obtained.

Rabbit 17: Given from 2 to 4 grains of peralga daily for forty-five days, producing no effect on the leukocyte count.

Rabbit 18: Given from 2 to 4 grains of dial daily for forty-five days, producing no effect on the leukocyte count.

CONCLUSIONS

1. Agranulocytosis is a clinical entity whose pathology is probably primary in the bone marrow, followed by sepsis which may be either local or general, or followed by no evidence of sepsis.

2. Subcutaneous injections of benzene and olive oil (if given in sufficiently small doses, so as not to affect the erythroblastic tissues) resulted in the development of clinical agranulocytosis in rabbits. The smaller the dose, the more selective became the affinity for the myelocytic tissues. The course of the condition seemed to be similar to that seen in the human, that is, first a neutropenia, then generalized infection from organisms already present, or from organisms introduced.

3. Agranulocytosis with subsequent infection and without infection was produced by the subcutaneous injection of benzene without olive oil and also by the intraperitoneal injection of benzene.

4. Benzene inhalations failed to depress the leukocyte count.

5. The intravenous injection of benzene even in small doses resulted in the immediate death of the animal, so it is probable that oxidation products of benzene are directly responsible for its leukocyte depressing properties.

6. Agranulocytosis can be produced with benzene in a rabbit having a leukocytosis.

7. A marked leukopenia was produced by the subcutaneous injection of ortho-oxybenzoic acid and by the intravenous injection of hydro-quinone.

8. The injection of animals by the subcutaneous and intravenous routes and the oral administration of the following substances failed to depress the leukocyte count: amidopyrine, phenacatine, peralga, dial, resorcinol, pyrocatechin, orthocresol, metacresol, paracresol, phenol, para-oxybenzoic acid, meta-oxybenzoic acid, 50 per cent alcohol.

9. Skin application and subcutaneous injection of the following substances failed to depress the leukocyte count: benzene cleaner, aniline, paranitraniline, para red dye, beta-naphthol.

10. The daily intravenous injection of various organisms isolated from the blood stream of patients with agranulocytosis failed to depress the leukocyte count.

11. The etiology of agranulocytosis is unknown, but the benzene ring must be strongly considered.

12. Those who have the opportunity to study this disease should direct their attention to a careful history of possible contact with granulopenic producing substances, bearing in mind that when the patient consults the physician, he presents the terminal stage of a disease that possibly began months before.

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DISCUSSION

T. H. BOUGHTON, Akron, Ohio: While I cannot doubt the experimental production of an agranulocytic syndrome by means of benzene, I greatly doubt the clinical significance of benzene in this condition. We are conducting a large-scale clinical experiment in this field. Due to the concentration of the rubber industry in and around Akron, and the very general employment of benzene in the factories we see a great many cases of benzene poisoning, probably more than at any other place in this country. Yet in spite of constant watchfulness, we have found only two cases of agranulocytosis, neither of them in persons subjected to any form of industrial poisoning.

Furthermore, the blood findings are totally different. Benzene poisoning gives the picture of aplastic anemia: very low red count, a color index of one, a moderate leukopenia, and a normal differential. Only a small proportion of the workers exposed to benzene ever develop benzene poisoning. If benzene could produce agranulocytosis, it seems likely that some of these workers experiencing a sub-toxic exposure would develop the syndrome, but we have not found them, and all persons in our factories exposed to benzene are subjected to frequent blood examination.

A SIMPLE MEDIUM FOR THE ISOLATION OF BACTERIA OF THE TYPHOID AND DYSENTERY GROUPS

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In laboratory cultures at the Philadelphia General Hospital for the past six years, the following medium for the isolation of bacteria of the typhoid and dysentery groups has been found to be of advantage over the Endo agar medium now generally used. The medium consists of the following ingredients:

Beef extract	3 grams
Peptone	10 grams
Sodium chloride	5 grams
Agar	25 grams
Lactose	10 grams
Water	1000 cc.

Heat until constituents are dissolved and filter through cotton and gauze. Place in flasks or tubes of convenient size and sterilize in the autoclave at fifteen pounds pressure for fifteen minutes.

Indicator: Dissolve 0.25 gram of brom-thymol-blue in 100 cc. of 95 per cent ethyl alcohol. Add 2 cc. of the indicator to 100 cc. of medium just before plates are poured.

The plated medium is light green in color and remains suitable for use over a period of weeks if stored in the ice-box. A loopful of emulsified feces or of the sediment of centrifugalized urine is streaked over the surface of the medium. After eighteen to twenty-four hours incubation at 37°C., the lactose-fermenting microörganisms produce yellow colonies and non-lactose-fermenters appear as blue colonies. A zone of the medium adjacent to the colonies likewise assumes these colors respectively.

The medium keeps indefinitely and is not affected by light. The tinted colonies are shaply defined and differentiated. Each

retains its particular color, even though they may occur adjacently on the plates.

Brom-thymol-blue exhibits no inhibitory action on bacteria of the colon-typhoid-dysentery groups, therefore these microorganisms grow as readily on this medium as they do on plain lactose-agar. This feature makes the medium especially serviceable for the primary isolation of bacteria belonging to the dysentery group.

A PRECIPITIN TEST FOR THE DIAGNOSIS OF MENINGITIS

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It is obviously of importance in the treatment of infections of the meninges that the laboratory diagnosis be made in the shortest possible time and that the commercial serum possessing the greatest specificity for the particular strain of microörganism causing the infection be selected for treatment.

For the past year, in the routine testing of spinal fluid from patients suffering from meningitis, we have employed a precipitin test on the clear spinal fluid after the bacteria and other cells have been removed by centrifugalization. We have proceeded on the assumption that in the spinal fluid of meningitic patients there is present a soluble precipitable substance (precipitinogen), derived from the invading microörganism, which is thrown out of solution when the spinal fluid is mixed with an immune serum containing precipitins for the particular strain causing the infection.

TECHNIQUE OF TEST

Spinal fluid from patients with suspected meningitis is centrifugalized at high speed for twenty minutes. The clear supernatant liquid is removed to another test tube by means of a capillary pipette. Smears and cultures may then be prepared from the sediment in the usual manner. From 0.3 to 0.5 cc. of each available commercial or stock serum is placed in small test tubes. An equal volume of supernatant fluid, which has been separated from the spinal fluid sediment, is layered over the serum by means of a capillary pipette. In less than twenty minutes, the precipitinogen in the spinal fluid will unite with any homologous precipitins in the serum to form a delicate but distinct white ring at the line of contact (Figs 1 and 2). After standing over night at room temperature, a precipitate is thrown to the bottom of the tube (or tubes) which showed an immediate ring test. The amount of precipitation may be graded

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from + to ++++. Control tests using normal spinal fluids failed to give either the white ring or the precipitate.

The test is not only a rapid and satisfactory method of determining the etiology of the meningitis (meningococcic, pneumo-

X   Y   Z
MENINGOCOCCIC SPINAL FLUID

X   Y   Z
NORMAL SPINAL FLUID

FIG. 1. SPINAL FLUID PRECIPITIN TEST WITH COMMERCIAL ANTIMENINGOCOCCIC SERUMS (X, Y, Z) IN A CASE OF MENINGOCOCCIC MENINGITIS

Appearance after standing twenty minutes. A precipitate forms which sinks to the bottom of the tube after eighteen hours.

Type I   Type II   Type III   Saline
PNEUMOCOCCIC SPINAL FLUID
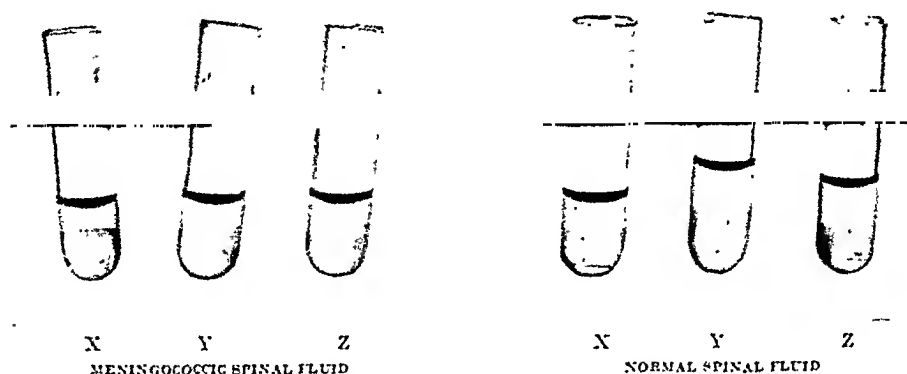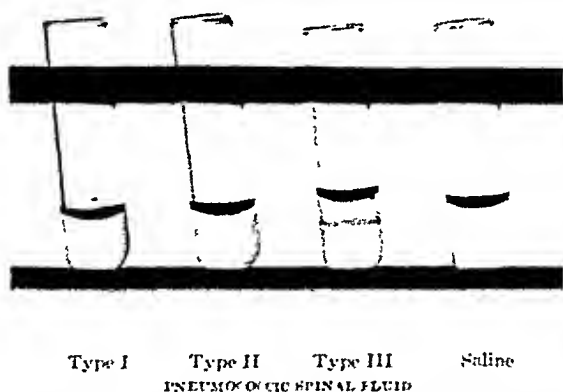
FIG. 2. SPINAL FLUID PRECIPITIN TEST WITH ANTIPNEUMOCOCCIC SERUMS IN A CASE OF PNEUMOCOCCIC MENINGITIS

Appearance after standing twenty minutes. A precipitate forms which sinks to the bottom of the tube after eighteen hours.

coccic, et cetera) but the density of the ring and the amount of the precipitate indicate which of the commercial serums available

TABLE 1

Results of Precipitin and Agglutinin Tests on 16 Spinal Fluids from Cases of Meningococcic Meningitis

NO.	PRECIPITIN TESTS USING UNDILUTED COMMERCIAL ANTIMENINGOCOCCIC SERA			AGGLUTININ TESTS USING UNDILUTED COMMERCIAL ANTIMENINGOCOCCIC SERA		
	X	Y	Z	X	Y	Z
1	++++	+	+	1:80	—	—
2	++++	++	++	1:80	—	—
3	++++	—	—	1:40	—	—
4	++++	—	—	1:160	—	—
5	++	—	—	1:160	—	—
6	++++	++	—	1:640	1:80	—
7	++++	+	+	1:160	1:40	1:20
8	++++	++	+	1:320	—	+
9	++++	—	—	1:160	—	—
10	++++	—	—	1:80	—	—
11	++++	++	—	1:320	1:20	—
12	+++	—	—	·1:80	1:20	—
13	++++	—	—	1:80	—	—
14	++++	—	+	1:640	1:80	1:80
15	++++	+	—	1:320	1:80	—
16	+++	—	—	1:320	—	—

Very strongly positive, ++++; strongly positive, +++; positive, ++; weakly positive, +; no precipitation or agglutination, (−). The figures represent the highest dilutions of commercial serums which agglutinated the isolated meningococci (in bouillon). Commercial Sera, −X, Y and Z. All controls were negative.

TABLE 2

Results of Bacteriologic and Serologic Tests on 31 Spinal Fluids

NO. OF SPINAL FLUID	RESULT OF SMEAR	RESULT OF CULTURE	PRECIPITIN TESTS			AGGLUTININ TESTS		
			Anti-meningococcic serum	Antipneumococcic serum	Anti-streptococcic serum	Anti-meningococcic serum	Antipneumococcic serum	Anti-streptococcic serum
15	Meningococcus	Meningococcus	Pos.	Neg.	Neg.	Pos.	Neg.	Neg.
5	Meningococcus	No growth	Pos.	Neg.	Neg.	No agglutinin tests possible		
1	Pneumococcus	Pneumococcus	Neg.	Pos. Type III	Neg.	Neg.	Pos. Type III	Neg.
1	Streptococcus	Streptococcus	Neg.	Neg.	Neg.*	Neg.	Neg.	Neg.*
9 (normal)	Negative	Negative	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.

* No homologous serum available.

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possesses the highest precipitin titre for the particular strain of microörganism causing the infection (table 1). Agglutination tests performed with the same serums and broth cultures of the isolated organisms have verified the results of the precipitin tests in each case tried (table 2). In five cases studied, in which the precipitin tests were positive, the agglutination tests could not be performed because the cultures were negative. In such cases the precipitin test possesses a distinct advantage.

SUMMARY AND CONCLUSIONS

A rapid and accurate precipitin test for use in the diagnosis of meningitis is described.

The precipitin test on meningitic spinal fluids renders possible the reporting of the causative organism and also the commercial serum most suitable for treatment within thirty minutes after the specimen reaches the laboratory.

The precipitin tests have been checked with agglutination tests whenever possible and results have agreed consistently.

In five cases studied in which the cultures were negative, thus rendering an agglutination test impossible, the precipitin test was positive. This fact and the possibility of returning an almost immediate report are advantages of the precipitin test in the diagnosis of meningitis that justify its use in the routine examination of spinal fluid in cases of suspected meningitis.

AN ANALYSIS OF 1535 AUTOPSIES*

OSBORNE ALLEN BRINES

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The following report is based on 1535 consecutive autopsies performed at Receiving Hospital during the past four years. The prevalence of various fatal diseases in this summary is doubtless influenced by prevailing hospital conditions. Receiving Hospital is a general hospital with a daily census fluctuating between 650 and 875 patients, the variation being due principally to conditions on the psychopathic service where there are between 200 and 400 patients. All other services are represented except obstetrical and contagious; the pediatric department is small. The main services, in addition to the psychiatric, are general surgery, general medicine and gynecology. It must be expected that the age of patients coming to autopsy in this series is higher than in most general hospitals.

Although Receiving Hospital maintains the principal emergency station in Detroit very few accident cases come to autopsy because most of such cases come under the jurisdiction of the Coroner and are immediately removed to the County Morgue either because of the medicolegal aspects presented or because the period of hospitalization was less than thirty-six hours.

I realize that the same autopsy material might easily yield different results in the hands of some other reviewer and it is not unlikely that different conclusions might be reached by the same individual a few years later due to changing view points and interpretations. It is also easily possible that a similar survey of autopsy material in another general hospital in the same community would present a totally different distribution of types of disease because of different clinical or staff conditions.

Seventy-six per cent of these autopsies were performed on males and there were twice as many white males as colored. Sixty per cent of the females were white. The average age of all patients was 42.7 years; that of white males 46.2, white females 41.1, colored males 39, and colored females 35.7. Eleven infants

TABLE 1

MAJOR LESIONS ENCOUNTERED AT AUTOPSY*

	NUMBER	PER CENT
Cardiac disease	315	20.0
Tuberculosis	223	15.0
Pneumonia	216	14.0
Renal disease	208	14.0
Syphilis	129	8.4
Malignancy	127	8.0
Peritonitis	94	6.0
Intracranial hemorrhage	90	5.9
Meningitis	64	4.2
Hepatic disease	48	3.1
Brain infarction	30	2.0
Septicemia	27	1.8
Intestinal obstruction	26	1.8
Diabetes	19	1.2
Anemia	12	
Intracranial abscess	10	
Ulcerative colitis	9	
Hemorrhage	7	
Thyrotoxicosis	6	
Splenic disease	5	
Addison's disease	4	
Cause undetermined	109	7.0

* In some instances more than one major lesion was found at autopsy or two classifications were possible for example, heart disease and syphilis, cardiac and renal disease, tuberculosis and peritonitis, et cetera.

whose age was less than one year and two fetuses were not counted in arriving at this average. Considering the high percentage of middle aged and elderly males, the unusually high incidence of some diseases and the low incidence of others is not surprising.

Of the 1535 cases, the cranial cavity was examined in 1070 cases, or 70 per cent. When a well established clinical diagnosis ruled

out the probability of intracranial lesions the examination of the head was omitted. It is not likely that many lesions were missed as the result of this practice. Table 1 illustrates the distribution of major fatal lesions. Cardiac lesions head the list as the greatest cause of death in this series. Table 2 gives the total number and the different types of cardiac cases encountered. I realize that the term "hypertensive heart" might elicit critisism from pathologists for being too clinical, but I have attempted to correlate clinical and pathological findings in arriving at a final diagnosis in order to make results more valuable to the clinical staff. In the hypertensive group were placed those cases of

TABLE 2

CARDIAC DISEASE

315 cases or 20 per cent of all autopsies

	NUMBER	PER CENT
Hypertensive heart	131	40
Coronary sclerosis	46	15
Bacterial endocarditis	39	13
Rheumatic heart	34	11
Pericarditis	27	9
Syphilis	22	6
Coronary occlusion	16	5
Myocarditis (active)	8	
Hemopericardium	2	

marked left ventricular hypertrophy with no valvular lesion and with coronary sclerosis of no, or only secondary, importance. In 15 per cent, or forty-six of the heart cases, coronary sclerosis without demonstrable occlusion was the most important lesion, and the underlying factor in the death of the patient. Perhaps these two groups should have been combined because it is often impossible from the autopsy material alone to classify accurately and separate hypertensive heart disease and coronary sclerosis.

In twelve of the twenty-seven cases of pericarditis there was suppuration and nearly all of these were due to pneumococcic infection which was the result of pleural extension. There were eight cases of adhesive pericarditis which were non-rheumatic,

nearly all of which were associated with cardiac hypertrophy, secondary myocardial degeneration and final cardiac failure. The remaining seven of the twenty-seven cases were cases of tuberculous pericarditis.

Six of the cases of myocarditis were presumably bacterial while the remaining two were suspected of being syphilitic, although spirochetes were not demonstrated. Of the sixteen instances of coronary' occlusion, twelve were old, two having resulted in left ventricular aneurism; the remaining four were cases of recent coronary thrombosis. This is a surprisingly low number of cases of coronary occlusion but because of the suddenness of death this type of case is quite likely not to come to autopsy at the hospital. In the sixteen cases of coronary occlusion there was no reason for suspecting syphilitic infection in thirteen, whereas in the remaining three there was either clinical, histological or serological proof of syphilis.

The high incidence of advanced renal arteriosclerosis associated with hypertensive heart disease is instructive (table 3); as far as could be ascertained, sclerosis of the large renal vessels occurred about as frequently as sclerosis of the arterioles. The average age of patients coming to necropsy with hypertensive heart disease was fifty years and 90 per cent occurred in males. No other type of cardiac disease seemed to be greatly influenced by the sex of the patient. In two cases of marked diffuse cardiac hypertrophy, the cause could not be discovered.

In the thirty-four cases of rheumatic heart infection, the inflammatory reaction was in a chronic stage in thirty. Table 3 also gives the complications of rheumatic carditis. The average age of the patients dying of rheumatic heart infection was thirty-eight years and 73 per cent were males; this was the percentage of males in all autopsies. Adhesive pericarditis complicated rheumatic carditis eleven times or 30 per cent which is a much lower figure than was anticipated.

There were two cases of vegetative tricuspid valvulitis, one of which was due to gonorrheal infection. The average age of the patients dying from bacterial endocarditis was forty-three years and 75 per cent were males.

Renal arteriosclerosis was present in only five cases, or 9 per cent of coronary sclerosis (table 3) as contrasted to 82 per cent in hypertensive heart disease.

The position is sometimes taken that many cases of aortic stenosis which is usually attributed to arteriosclerosis are really

TABLE 3
Cardiac Disease Complications

	NUMBER	PER CENT
Hypertensive heart (131 cases)		
Renal arteriosclerosis................................	108	82
Coronary sclerosis....................................	12	10
Syphilitic aortitis....................................	3	
Unclassified...	8	
Coronary sclerosis (46 cases)		
Cardiac hypertrophy..................................	23	50
Syphilitic aortitis....................................	4	8
Renal arteriosclerosis................................	5	9
Aortic stenosis......................................	8	17
Unclassified...	6	
Rheumatic heart (34 cases)		
Mitral stenosis......................................	14	41
Aortic and mitral stenosis............................	9	27
Aortic stenosis......................................	3	9
Unclassified...	8	
Bacterial endocarditis (39 cases)		
Mitral valve...	20	51
Aortic valve...	11	30
Aortic and mitral....................................	6	16
Tricuspid..	2	

the end results of rheumatic infection. Considering the age, however, of these eight patients together with the absence of inflammatory changes, the marked coronary sclerosis present and the low incidence of aortic stenosis found in typical rheumatic heart disease, it would seem rather poor logic to consider aortic

stenosis, which is found associated with coronary sclerosis, in individuals of middle age or past, as being on a rheumatic basis. The average age for this group was fifty-eight years with 75 per cent males.

The wide range in age incidence in all types of heart disease contributes little to facilitate clinical diagnoses. In the hypertensive group there were five patients aged between twenty and thirty years, three of these cases being associated with glomerulonephritis; there were twenty patients between thirty and forty years, while ten patients were aged seventy or more years. Fourteen of the thirty-three cases of rheumatic heart disease occurred

<div align="center">

TABLE 4

TUBERCULOSIS*

223 cases or 15 per cent

</div>

Pulmonary	169 cases
Bilateral	146
Left only	13
Right only	10
Generalized miliary	52 cases
Tuberculous meningitis	11 cases
Tuberculous peritonitis	10 cases
Tuberculous pancreatitis	5 cases
Tuberculous myocarditis	3 cases

* Some cases had more than one tuberculous lesion; for instance, one case may be listed under miliary, pulmonary and peritonitis. The greater number of cases than lesions is thus accounted for.

in patients between the ages of forty and fifty years whereas three patients were older. One case of advanced coronary sclerosis was found in a colored male aged twenty-five years; five other cases were in patients in the next decade. In two cases of coronary thrombosis the patients were aged thirty-three and thirty-five years.

A patent foramen ovale was found in two infants and in a white male aged forty-six years. A patent ductus arteriosus was found in an eleven months old child.

Tuberculosis accounted for 223 cases, or 15 per cent, of all deaths, and ranked second as a fatal disease. Table 4 may be

consulted for the distribution of lesions. In fifty-eight cases, or 34 per cent, of the pulmonary tuberculosis there was severe terminal tuberculous infection of the liver while infection of the myocardium was found only three times and of the pancreas five times.

Pneumonia accounted for the third largest number of deaths. In this group there were 216 cases, or 14 per cent, of all deaths. One hundred eighty-eight of these were lobar and twenty-five bronchopneumonia. Of the 171 cases of lobar pheumonia in which the side involved was recorded, there were thirty-seven, or 22 per cent, in which the lesion was bilateral, thirty-nine, or 23 per cent, with left side involvement only and ninety-five, or 55 per cent, in which only the right lung was involved. In ten additional cases suppurative pleurisy accounted for death.

Renal disease ranked fourth as a fatal lesion. Table 5 shows the incidence of the various types of renal lesions. Eighteen of the twenty cases of glomerulonephritis could readily be classified as chronic. In three instances hypertensive heart disease was associated with and apparently the result of glomerulonephritis. In four, or 20 per cent, of the cases of glomerulonephritis there was rheumatic heart disease and a common etiology in these instances cannot but be considered. In five additional cases of glomerulonephritis there was superimposed renal arteriosclerosis. One patient with chronic glomerulonephritis died of eclampsia.

Fourteen, or 7.4 per cent, of the cases of non-suppurative renal disease presented a clear cut chronic or subacute inflammatory lesion apparently the result of bacterial infection. The microscopical picture was an infiltration of inflammatory cells which were chiefly lymphocytes with a few polymorphonuclears, plasma cells and eosinophiles, associated with some connective tissue hyperplasia. In half of these the inflammatory picture was diffusely distributed whereas in the other half there was focal distribution. These I designated as stromal, or interstitial nephritis; those with the diffuse distribution roughly resembled the nephritis following scarlet fever although the history in none of the cases revealed previous acute infection. It is further interesting to note that in four of these twelve cases jaundice was an outstand-

ing symptom and that a definite active chronic hepatitis was usually present.

The complications of the 131 cases of vascular nephritis were distributed as follows: sixty-eight, or 52 per cent, were associated with hypertensive heart disease and fifteen, or 11 per cent, with coronary sclerosis. In other words, about half of the cases of renal arteriosclerosis occurred without hypertensive heart disease whereas the latter was nearly always associated with severe vascular disease of the kidney. In eight, or 6 per cent, of the cases of renal arteriosclerosis there was intracranial hemorrhage and in nine additional cases there was both hypertensive heart disease and cerebral hemorrhage. No cases of classical nephrosis were encountered in this series. Congenital polycystic kidneys

TABLE 5

DISTRIBUTION OF RENAL DISEASE

208 cases or 13 per cent

	NUMBER	PER CENT
Renal arteriosclerosis	131	63.0
Glomerulonephritis	20	10.0
Suppurative	19	9.0
Stromal nephritis	14	7.0
Glomerular and arteriosclerosis	5	2.4

were found in two cases and horseshoe kidney was found twice. There were six additional cases of severe pyogenic infection of the lower urinary tract resulting in the death of the patient.

During the past year solitary cyst of the kidney was an accidental autopsy finding in seven cases. These cysts ranged in size from 5 to 18 cm. in diameter. This suggests that if more pathologists would report solitary cysts of the kidney encountered routinely, the lesion would lose, to a large extent, some of the reputation which it now enjoys of being a rare lesion.

Syphilis assumed fifth place with 129 fatal cases, or 8.4 per cent, of all deaths. Table 6 shows the type of fatal syphilitic lesion present. Eighty-two, or 38 per cent, of all cases of syphilis, or 64 per cent of all fatal syphilis, showed evidence of central nervous

system involvement and of these eighty-two cases, fifty-nine, or 79 per cent, were diagnosed syphilitic meningo-encephalitis, the clinical diagnosis of which was usually paresis. Gumma of the brain was found twice and of the liver three times. Of the eighty-two cases of cerebral syphilis there was no evidence of syphilitic aortitis in twenty-seven, or 32 per cent. The large number of cases of neurosyphilis is, of course, due to the large number of psychopathic patients housed in this hospital and would not be encountered in the autopsy work of the average general hospital.

TABLE 6
SYPHILITIC INFECTION
216 cases

Neurosyphilis	82 (38 per cent)
(Meningoencephalitis—59)	
Neurosyphilis without aortic lesion	27 cases
Thoracic aneurism	19 (9 per cent)
Syphilitic aortitis	12 per cent of all autopsies

FATAL CASES OF SYPHILIS
129 or 8.4 per cent of all autopsies

Neurosyphilis	82 cases
Aortic valve lesion	22 cases
Cerebral hemorrhage (syphilitic)	8 cases
Cerebral infarction (syphilitic)	8 cases
Ruptured aneurism	6 cases
Hereditary syphilis	5 cases

Deducting the eighty-two cases of death from neurosyphilis which would bring conditions roughly down to the level encountered in most general hospitals, syphilis would then be reduced from fifth to eleventh place as a cause of death or 3 per cent of all autopsies instead of 8.4 per cent.

In 216, or 15 per cent, of all autopsies there was histopathological evidence of syphilis. Syphilitic aortitis was found in 184 cases, or 12 per cent, of all autopsies. Aortic aneurism on a syphilitic basis was found nineteen times, or in 10 per cent of the cases of syphilitic aortitis; only six of the aneurisms had ruptured.

There was syphilitic heart disease (aortic valve involvement) in twenty-two cases, or 12 per cent, of 184 cases of syphilitic aortitis. In three additional instances there was combined aortic aneurism and aortic regurgitation on a syphilitic basis. Syphilitic aortitic infection (ruptured aneurism or aortic regurgitation or both) caused death in thirty-one instances, or 17 per cent, of the cases of syphilitic aortitis which was only 2 per cent of all the cases autopsied. Routine Wassermann and Kahn tests in this hospital on patients admitted averaged 13.7 per cent positive.

A study of a group of 105 cases of syphilitic aortitis showed that the diagnosis was made at the autopsy table in only fifty-

TABLE 7

MALIGNANT DISEASE

127 cases (8 per cent)

Carcinoma...	93 (74 per cent)
Sarcoma...	9 cases
Melanoma...	4 cases
Hodgkin's disease.......................................	4 cases
Glioma..	4 cases
Leukemia..	4 cases
Meningioma...	4 cases
Teratoma of testis.......................................	2 cases
Pituitary tumor...	2 cases
Auditory nerve tumor...................................	1 case

eight instances, or 53 per cent. The difficulty in making a diagnosis of syphilitic aortitis from gross specimens was apparently due, at least to some extent, to the high age average of the postmortem subjects and therefore to the presence of superimposed aortic atherosclerosis. In the nineteen cases of aortic aneurism, cardiac hypertrophy occurred in six, or 32 per cent. Of the 184 cases of syphilitic aortitis, active tuberculous infection was found twenty times, or 11 per cent, which was 4 per cent less than the incidence of tuberculosis in all cases autopsied. It should be stated that nearly all of the syphilitic patients in this hospital are untreated. There were five cases of hereditary syphilis, the ages being twelve days, three months, six months, two years and three years.

Malignant disease ranked sixth as a cause of death with 127 cases, or 8 per cent. Table 7 presents the frequency with which the various types of malignancy were encountered.

Carcinoma of the stomach occurred twenty-five times with metastasis to the liver in thirteen of these cases. Twenty-two of these twenty-five cases occurred in white males and two in colored males. Carcinoma of the stomach appeared to follow an old peptic ulcer in two cases, or 8 per cent. Primary carcinoma of the lung and of the large intestine came next in order of frequency, there being eleven of each. Of the eleven carcinomas

TABLE 8

CARCINOMA OR 93 CASES

Stomach	25 cases
Lung	11 cases
Intestine	11 cases
Uterus {cervix 2 / body 7}	9 cases
Prostate	8 cases
Esophagus	6 cases
Liver	5 cases
Pancreas	5 cases
Kidney	4 cases
Thyroid	3 cases
Breast	2 cases
Bladder	2 cases
Brain	1 case
Gall bladder	1 case

of the lung, six were the small spindle, oat cell or columnar cell type, while three were squamous and two adenocarcinoma. Of the eleven cases of carcinoma of the intestine, seven occurred in the rectum and two of these were squamous. In Table 8 there are additional figures relative to the location of cancerous lesions. Of the three cases of carcinoma of the bladder, skeletal metastasis was found in two. Primary carcinoma of the liver was found five times, two of which were cholangiomas and three hepatomas. There was one carcinoma of the gall bladder.

In six carcinomas of the esophagus, two showed no evidence of metastasis. There were four cases of carcinoma of the kidney,

two of which were interesting in that there was bladder implantation. In one case multiple malignant adenomas were found in both kidneys. Another papillary carcinoma of the kidney had metastasized to the regional lymph nodes and to the opposite adrenal. There were three cases of carcinoma of the thyroid gland, one of which contained large areas of spindle cells. There were five cases of carcinoma of the pancreas, three occurring in the head and two in the tail. There were two patients with carcinoma of the breast, both of whom had had breast amputations. There was one carcinoma of the cerebellopontine angle, the source of which was undetermined. There was only one instance of a sarcoma growing in a uterine myoma which is remarkable in that during this period 443 specimens of uterine myoma were examined in the laboratory, but only seven of these showed a suspicion of early malignancy microscopically.

Acute generalized peritonitis was encountered ninety-four times, or 6 per cent of all autopsies, and of the ninety-four cases thirty-seven, or 40 per cent, followed operation. No cases of acute gangrenous or ruptured appendix or ruptured peptic ulcer were included in this 40 per cent whether operation had been performed or not. Of the thirty-nine cases of postoperative peritonitis seventeen, or 44 per cent, followed pelvic surgery. In most of these the inflammatory activity of the tissue removed exceeded the postoperative anticipation of the surgeon. It might be added that the greatest conservatism is practiced on the gynecological service of this hospital and that in spite of this conservatism, pelvic surgery heads the list as a cause of fatal postoperative peritonitis. This is a point which many surgeons apparently do not fully appreciate. Twenty-six per cent, or ten cases, of postoperative peritonitis followed the removal of non-suppurative appendices while six cases followed gastro-intestinal operations and four followed gall bladder surgery. Hernial repair and abdominal paracentesis each contributed one case of postoperative peritonitis. Of the remaining fifty-five cases of generalized peritonitis which did not follow operation, or in which operation was performed only after rupture of a viscus had occurred, twenty-one, or 38 per cent, resulted from an acute

infection or gangrene of the appendix with or without perforation, while eleven or 22 per cent, resulted from perforation of a peptic ulcer which were about equally divided between gastric and duodenal. One case of gastric ulcer occurred in a syphilitic patient and there was impressive histopathological evidence of syphilis in the ulcer base but spirochetes could not be demonstrated.

Intracranial hemorrhage was a fatal lesion ninety times, or 5.9 per cent, of all autopsies. Only four of these were associated with skull fracture and only one was produced by bullet wound. It has been previously pointed out that the low percentage of traumatic cases was due to the fact that the majority of such cases are taken care of by the coroner. In ninety cases of brain hemorrhage thirty-eight, or 42 per cent, appeared to be definitely on an arteriosclerotic basis and in three of these ages were thirty-three, thirty-five and twenty-nine years. Six or 7 per cent of the brain hemorrhage cases had hypertensive heart disease. Cerebral syphilis, however, was present in the surprisingly low number of eight, or 9 per cent. There were eight cases of brain hemorrhage, the cause of which could not be definitely determined. In three of these encephalitic lethargica was probably present. In others there was a question of syphilis while in one case there was an history of alcoholism. These cases of intracranial hemorrhage will be made a subject of a special study to be reported later. A rough survey, however, seems to indicate that an age of fifty years is not a valuable clinical dividing line between brain hemorrhage on an arteriosclerotic basis and that on a syphilitic basis.

There were sixty-four cases of meningitis, or 4.2 per cent, of all cases. The etiology was undetermined in twenty, seventeen were meningococcic, thirteen pneumococcic and five staphylococcic. Mastoid or middle ear infection accounted for nine of the fifty-five cases of suppurative meningitis. In two instances purulent meningitis was superimposed on syphilitic meningitis. Besides the fifty-five cases of suppurative meningitis, there were three of tuberculous meningeal infection and three of chronic leptomeningitis, the etiology of which could not be determined.

Pathologic lesions of the liver probably deserve a more prominent place as causes of death than statistics would indicate; how-

ever, death was attributed to liver disease in forty-eight cases, or 3.1 per cent. There were twenty-six cases of cirrhosis of the liver, twenty-four of which were portal, one biliary and one toxic. This hospital has a large number of patients which are classified as chronic alcoholics and the low incidence of alcoholic cirrhosis of only six cases a year appears surprisingly small. There were twelve instances of liver abscess, nine of which were multiple and three of these were secondary to appendiceal infection. Of the three solitary abscesses of the liver, two were amebic. Four cases of acute yellow atrophy of the liver occurred. There were six interesting cases of severe subacute diffuse hepatitis, three of which were associated with severe gall bladder infection. In two of these there was conspicuous atrophy and necrosis which would classify this lesion very close to subacute yellow atrophy.

Toxic degeneration of the liver, as a secondary or terminal condition, was noted in 104 cases. Only the severe cases, with parenchymatous damage and extensive intracellular infiltration of fat which had progressed to the point where it seemed reasonable to believe that the function of the liver was seriously impaired were included. Further classifying these cases of liver degeneration I found that the largest number (twenty-one, or 20 per cent) were associated with "alcoholic" poisoning. The toxic agent in these cases, however, was probably not the ethyl alcohol but accidental poisons peculiar to some beverages. Sixteen additional cases of severe liver damage were associated with lobar pneumonia, fifteen with tuberculosis, ten with acute peritonitis and seven with brain hemorrhage. In six cases there was no apparent cause for the liver damage while three additional cases were infants where the pathology was not clear cut. Chemical poisoning was responsible for four more, two of which were from morphine and one from lysol. There can be little doubt that the liver damage resulting from bacterial and chemical toxins is a big mortality factor.

Cholelithiasis was an incidental finding, apparently not producing symptoms, in thirty-six cases, or 2.4 per cent. There were three cases of hepatic duct calculi. These soft, easily fragmented masses between 2 and 3 cm. in diameter were found in both the

hepatic and common duct. In spite of these impacted calculi in the common duct there was no appreciable jaundice in any of these three cases.

Definite lesions of the spleen were rather rare. There was one case of Banti's disease, two of traumatic rupture, two of amyloid disease, while in one case extensive tuberculosis was found with the spleen the only organ involved.

There were thirty cases of brain softening without hemorrhage, constituting 2 per cent of all autopsies. Fifteen of these were on an arteriosclerotic basis, eight the result of syphilitic endarteritis, four associated with aneurism of the intracranial vessels and in three the cause was not determined.

General septicemia was a definite diagnosis in twenty-seven cases, eight of which were staphylococcic.

In twenty-six cases of intestinal obstruction which had resulted in the death of the patient, fourteen were postoperative and four of these followed appendectomy, four followed hernia repair and two after pelvic operations. Of the twelve cases which did not follow operation, five were due to strangulated hernia and three to old pelvic infection.

Nineteen cases of diabetes mellitus occurred in eleven females and in eight males with an average age incidence of forty-six years. The earliest mortality from diatetes occurred at the age of fifteen years and the oldest patient was seventy. Microscopic changes in the pancreas could be detected in only six, or 35 per cent. Gangrene of the extremities was found in three of the nineteen cases.

Primary anemia was the cause of death in twelve cases and six of these were typical cases of uncomplicated pernicious anemia. One case was diagnosed pernicious anemia but there was a bacterial endocarditis and in another case the anemia was associated with syphilis. In two other cases a diagnosis of atypical pernicious anemia was made and one of these patients was a twenty-three year old white male. In two other cases the cause of the anemia was undetermined. A diagnosis of pernicious anemia, therefore, could really be made in only six of these twelve cases.

Brain abscess was found ten times and five of these were asso-

ciated with mastoid infection while in three others there was other sinus infection. The two remaining cases followed hernia operations.

Hemorrhage as a cause of death was found seven times, three of which were postoperative. One was traumatic abdominal hemorrhage and another was due to omental and mesenteric thrombosis. There was one case of hemorrhage of the new born. Epistaxis was fatal in one case of hereditary syphilis at the age of three years.

There were six deaths from thyrotoxicosis.

Second and third degree burns accounted for death in five patients. Here it is interesting to note that a severe active myocarditis was found in two cases, while in only one could definite evidence of renal damage be demonstrated.

There were nine cases of ulcerative colitis, five of which were associated with hemorrhage.

A survey of fifty consecutive cases of cardiac hydrothorax revealed the unexpected information that fluid was found on both sides in thirty-seven cases, on the left side in nine cases and on the right side in only four. In the majority of the cases of bilateral hydrothorax it was definitely stated that the greatest amount of fluid was found on the left side. This is in direct contradiction to the usual text book information on this point.

There were four cases of Addison's disease, three of which were tuberculous and in two of these an old tuberculous focus could only be found in the lungs. The fourth case was one of amyloid disease involving not only the adrenal but also the myocardium, lungs, spleen and liver.

In 109, or 7 per cent, the cause of death could not be satisfactorily determined. Twenty-six of these patients were senile and twenty-four were psychopathic patients, four of whom were epileptic. There were twelve infants and in six postoperative deaths no organic lesions could be found which would satisfactorily explain death. Syphilis was present in five cases and five others had a definite history of alcoholism. In three there was slight thymic enlargement but in none did a diagnosis of status thymicolymphaticus seem appropriate. In two cases there was

clinical evidence of myocardial failure but no lesion of the heart was demonstrated.

The gross and microscopic diagnosis failed to agree in ninety-five instances. In forty of these there was practically complete disagreement. It was anticipated that some valuable deductions could be made from a careful study of these discrepancies. These errors in gross diagnosis were found to be so widely distributed over such a large number of pathological conditions that very little could be salvaged from such a long and miscellaneous list. The inability accurately to diagnose cerebral syphilis without the aid of the microscope was probably the most common error. Confusing lobar pneumonia with tuberculous pneumonia and

TABLE 9

CAUSE OF DEATH UNDETERMINED

109 cases or 7 per cent

Senility	26 cases
Psychopathic	24 cases
Infants	12 cases
Postoperative	6 cases
Syphilis present	5 cases
History of alcoholism	5 cases
Enlarged thymus	3 cases
Clinical myocardial failure	2 cases

bronchopneumonia with acinar tuberculosis contributed some difficulty. Determining the correct type of non-suppurative kidney disease also seemed to be somewhat difficult. In the main, however, the gross diagnostic error was quite thinly spread over a large number of conditions.

UNUSUAL CASES

The unusual and interesting cases encountered are listed below:

Two cases of annular pancreas constituting the twenty-eighth and thirtieth cases of annular pancreas reported in the literature.

One case of spontaneous rupture of the liver due to infarction of the left lobe which was associated with suppurative pylephlebitis undetermined origin. There are apparently no cases of spontaneous rupture of the liver in the medical literature.

One patient died of rabies following a dog bite six weeks before.

Two cases of "idiopathic" dilatation of the esophagus or esophagectasia.

One case of complete absence of all genito-urinary apparatus on one side in a male. The adrenal which remained was markedly hemorrhagic (apoplexy of the adrenal).

In one case of melanoma, the liver weighed 5000 grams and was the only site of metastasis of melanoma of the retina.

In one case of generalized tuberculosis, active tuberculous infection, proved by bacterial stains, was found associated with primary carcinoma in the thyroid gland.

In a case of rheumatic heart disease with adhesive pericarditis coronary varices had developed, one of which had ruptured and a terminal hemopericardium had resulted.

There were two ruptured intracranial aneurisms involving the larger vessels (basilar and internal carotid arteries).

Rupture of an aneurism of the right common iliac artery on an arteriosclerotic basis with an unruptured aneurism of the left common iliac was found in a minister sixty-seven years of age.

There was a case of parathyroid adenoma with generalized osteitis fibrosa cystica and diffuse giant-cell tumor. In this case the patient died of uremia due to impaction of the renal pelves with insoluble calcium salts.

There was one case of paradoxical embolism with a patent foramen ovale and a saddle thrombus at the bifurcation of the abdominal aorta and one in the foramen ovale. The patient was forty-six years of age and there was an embryonal carcinoma of an undescended testis with general mestastasis.

One instance of congenital absence of a gall bladder was found.

Tuberculous endometritis was the only tuberculous lesion found in one case.

There was a case of thymoma which had metastasized to the liver, spleen, kidneys and vertebrae.

In one case a ruptured dissecting aneurism of the branch of the right anterior cerebral artery was found. There had been a severe blow on the head immediately over this area four years previously, which had not produced fracture of the skull. In the interval

the patient had had almost continual headaches, periods of dizziness, personality changes and fainted easily.

In one case hemopericardium was found due to perforation of the root of the pulmonary artery within the pericardial sac.   Immediately before death, the patient, a woman, had been hit with a framed picture thrown by her husband.   A narrow, finely pointed piece of glass 3 cm. in length had penetrated the chest wall to the right of the sternum.   The piece of glass had recoiled into the areolar tissue of the chest wall and was found with considerable difficulty.

Multiple malignant tumors were found in two cases.   In one a cerebral glioma was associated with carcinoma of the prostate. In the other case primary carcinoma of the pancreas was associated with hydronephroma of the right kidney.

Carcinoid tumor of the appendix and rectum occurred in the same individual.

SUMMARY

This analysis has been made without any attempt at strengthening or disproving any existing ideas.   The findings were quite incidental and comments were made wherever they were deemed appropriate.   It is obvious that no attempt has been made to account for the cause of death in all of the cases which came to autopsy.   It was intended that mention be made only of those cases which might possibly be instructive or interesting.

The writer was impressed by the frequent multiplicity of diseases at the time of death, a large number of instances being encountered of the simultaneous occurrance of three or four major diseases.   While this has been an interesting observation it has not been made a feature of this paper.

FOCAL CYCLIC GROWTH AS A FACTOR IN PRODUCTION OF NODULAR GOITER*

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As an introduction to the pathology of the nodular thyroid gland, some phases of its physiology should be considered, particularly the gland unit and its mode or products of secretion. The ultimate histologic unit of the gland is represented by the individual follicle composed of a sac lined by a single layer of thin, endothelial like epithelium, enclosing the colloid substance. The follicles do not branch or join others but are entirely discontinuous and are separate individual units as Rienhoff[3] has shown by a method of maceration and microdissection particularly applicable to the study of the individual follicle.

This histologic unit, as shown by Williamson and Pearse,[5] is concerned with two separate processes in the physiology of the thyroid gland:

1. The process of thyroid secretion which is active and associated with hyperplasia.
2. The process of accumulation of colloid which is passive and associated with involution.

These two processes follow each other and may be referred to as the first and second part of a functional cycle. During various periods of life either one of these two processes predominates with a resultant histological picture which is characteristic of the individual's age. The thyroid of the newly born is very active and composed of closely packed granular cells; this illustrates the process of secretion. Following the activity of infancy, the thyroid gland gradually assumes a resting stage with follicle formation and colloid retention illustrating the

* Read before the Tenth Annual Convention of the American Society of Clinical Pathologists. Philadelphia, Pennsylvania, June 7–9, 1931.

process of colloid accumulation. With the age of puberty the secretory phase of the gland again predominates sometimes so over shadowing the colloid phase that it exceeds the normal limits of activity, producing in late puberty or early adolescence what is usually termed "adolescent goiter." In later adolescence the gland again reverts to the resting or colloid stage which in adult life becomes more intensified.

These two processes occur as functional cycles in the thyroid mechanism and are the basis of the constant hyperplasia-involution process. When the body demands an increase in secretion a change in the histological picture called hyperplasia is the result and when these demands are met another histological change called involution occurs. With the latter, colloid storage with reversion to normal is seen.

When however these two processes do not follow each other in physiological order one or the other will dominate the picture with its characteristic findings. The functional cycle is then incomplete and the dominating process passes the physiological point. When this dominating process is finally counter-acted by the other, it has already passed into the abnormal state. This disproportion between these two cyclic processes at focal points is the basis of goiter nodulation as contended by Hertzler[2] and Graham.[1]

FOCAL AREAS OF INVOLUTION WITHIN A HYPERPLASTIC GOITER

The first process of physiological hyperplasia if too long continued, before the second process of involution occurs, causes the gland to become abnormally hyperplastic. A further continuation of this hyperplasia without counter-action by involution will result in a diffuse hyperplastic gland with the marked symptoms of the goiter which is clinically termed exophthalmic. At any period, usually late, during this hyperplastic state however, the first process of secretion may be arrested and followed by the second process of involution, either by medication or bodily adjustment.

With the arrest or counter-action, however, not all portions of the gland may be affected by the involution process and those

that are involved may not be acted upon to the same degree. Rienhoff[4] removed a portion of the thyroid in a small number of cases of severe Grave's disease, administered iodine for about two weeks and then removed the remaining portion of the gland. Prior to the use of iodine these glands presented the usual diffuse picture characteristic of what is clinically called exophthalmic goiter. After the use of iodine the diffuse picture was replaced by one composed of various sized, definitely circumscribed nodules. Microscopically, the change varied from a slight to a very marked degree. Even where only a slight change was noted, the dominant factor was the attempt of arrested growths with involution characterized by formation of circumscribed areas showing colloid instead of proliferating epithelium.

This involution is quite rapid when produced by the use of iodine but very slow, extending possibly over a period of years, when produced as a result of the natural exhaustion, or "burning out" process. In either case the hyperplastic cells of the follicles recede and there is formation of colloid which may later be replaced by fibrosis and regressive changes. The active process of secretion is being replaced by the passive process of involution and colloid storage. The follicles then become distended the intervening walls give way and several follicles are blended into one large colloid space. The fibrous septa surrounding this space are thickened and over a period of time forms a definitely encapsulated colloid area. The circumscribed tissue keeps going through the same uneven cycle until it becomes degenerated and with each successive step of involution the nodule becomes larger.

FOCAL AREAS OF HYPERPLASIA IN AN INVOLUTING GOITER

When the first process of secretion of a mildly hyperplastic goiter is counter-acted by the second process of involution a colloid goiter results. This colloid goiter is diffuse and smooth only when these two processes act with the same intensity over the entire gland. If, however, a single localized area of hyperplasia fails to become arrested and fails to recede to the colloid resting state, it remains as a focal hyperplastic area. Within this area there is a formation of new follicular structure with increasing

lack of orderly arrangement. Further proliferation within this localized area continues, causing it to increase in size and with increasing growth more and more pressure is exerted upon the surrounding tissue producing pressure necrosis about the periphery. In this immediate periphery atrophic changes occur and replacement fibrosis of the portions undergoing atrophy isolates this area of irregular hyperplasia with formation of the usual nodule.

EXAGGERATION OF BOTH PROCESSES

I have attempted, so far, to explain a probable factor in goiter nodule formation by showing that either the first process of secretion is overwhelmed by the second process of colloid accumulation or vice versa, that the colloid is sacrificed to secretion. There must be a third order of dysfunction of the thyroid gland in which both these processes are exaggerated with production of a temporary balanced hyperplasia. In this type the first process of hyperplasia, is not sufficiently arrested to cause a simple colloid goiter. Early in the disease however the exaggerated colloid process overcomes the secretory one at focal points. The principle involved in producing nodulation is similar to that in hyperplastic goiter or Grave's disease, except that the second process of colloid accumulation occurs early in the disease. Before the diffuse hyperplasia has had much opportunity to cause the colloid process to be sacrificed by the secretory one, the colloid accumulation occurs at focal points with production of nodules at these points.

In the thyroid gland where the first process of active secretion is counter-acted only in the later stages and at focal points by the second process of passive colloid accumulation there results focal nodulation within a diffuse hyperplastic goiter called Grave's disease, nodular exophthalmic goiter.

If the thyroid gland where the second process of passive colloid accumulation is sacrificed at focal points by the first process of active secretion there results a nodular colloid goiter, so-called non-toxic adenoma.

In the thyroid gland where there is evidence of both active and

passive disorder there results a nodular hyperplastic goiter or so-called toxic adenoma.

SUMMARY

1. There are two distinct processes in the physiology of the thyroid gland which produce a constant hyperplasia-involution cycle.

(a) Process of thyroid secretion—active with hyperplasia.

(b) Process of accumulation of colloid—passive with involution.

2. These two processes occur as functional cycles and when in disproportion produce nodulation.

(a) In diffuse hyperplastic goiter or Grave's disease, the process of secretion overwhelms the process of colloid storage. Finally, late in the disease, this colloid storage replaces the secretion at focal points with production of nodules.

(b) In nodular colloid goiter the process of colloid storage overwhelms the process of secretion. But at focal points the secretory phase continues and is not replaced by colloid storage with production of nodules.

(c) In nodular hyperplastic goiter there is over growth of both processes with colloid storage replacing thyroid secretion at focal points early in the disease with production of nodules.

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MODIFIED SILVER STAIN FOR TREPONEMA PALLIDUM

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Since the publication of the original Levaditi method of demonstrating the presence of *Treponema pallidum* in tissues, many modifications have been suggested, designed to make the effort less laborious and more uniform in result. Hitherto, however, there has been so much "trickiness" about the application of these procedures, or it has required so much time, or the chemical adjustments have been so intricate, that the ordinary laboratory worker has found considerable difficulty in obtaining successful results with any of them.

The technic described below has so far proved to be easy of application, rapid, and uniform and positive in results:

1. Fix in formalin, 10 per cent. Tissues fixed for many years have given positive results.
2. Embed in paraffin by the usual technic and cut sections about 5 microns in thickness. Sections cut after freezing would serve as well, if cut thin enough.
3. Remove paraffin in usual manner.
4. Immerse in nitric acid, 20 per cent, for ten minutes.
5. Wash well in tap water and rinse in distilled water.
6. Immerse in silver nitrate, 3 per cent, heated to 50°C., for fifteen minutes.
7. Place slides in petri dish, sections facing up, and pour over them gently the following solution, shaking dish until sections are light brown: 5 cc. of silver nitrate, 3 per cent, to which are added 20 cc. of gelatin 3 per cent, at 40°C., and 1 cc. of hydroquinone, 1 per cent. The hydroquinone must be added quickly and the resulting mixture must be used immediately and made fresh for each batch of slides.
8. Wash slides in distilled water at 50°C. to remove gelatin; then in tap water.
9. Immerse in 1 per cent formalin for two minutes.
10. Wash in distilled water.
11. Place in hyposulphite, 2 per cent, for two minutes.
12. Dip in tap water.
13. Dehydrate, clear, and mount in Canada balsam.

EDITORIAL

Medical Research—Clinical Investigation

Fortunately, or possibly unfortunately, the English language is extremely flexible and at times burdened with words. This very attribute may lead us into the error of extreme specialization even in choosing our views, not to mention titles, for discussion. The search for nature's truths has only changed its methods, but not its modes. Human nature is still its foundation. The inquisitive one—the disbeliever—tries for new information, and youth or age is no deterrent in this quest so long as physical and mental alertness are combined with the necessary fundamental knowledge.

Fundamental investigation does not limit its fields when there are men seeking knowledge and truth. Like religion, there may be many denominations, but its value depends upon the individual affected. Two most interesting contributions have just appeared, one from the pen of a surgeon on "What Place Has Research In The Hospital"[1] and the other from a clinician on "Clinical Investigation."[2] Both are timely and worthy of serious attention and thought; both are written to promote study in the broad field of medicine—one views the subject from the standpoint of the advantages to the patient, to the hospital, to the staff and for the advancement of medicine; the other analyzes clinical investigation in the light of a constitutional pledge to a society made twenty-three years ago and reviews the changes that have intervened and the development of a maturity of thought which has already characterized other biological sciences. Especially important is the broad view taken that, argumentation over the superiority of the descriptive method, including observa-

[1] Scott, W. J. Merle: What place has research in the hospitals? Bull. Am. Hosp. Assn., 5: 63–67. 1931.

[2] Blake, Francis G.: Clinical Investigation. Science, 74: 27–29. 1931.

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tion, analysis and deduction, as against the inductive, experimental method, or vice versa, is fruitless and narrow. Both methods are complementary halves of the whole and are, as expressed by Francis Bacon, "part of a double scale or ladder, ascendent and descendent, ascending from experiments to the invention of causes and descending from causes to the invention of new experiments." Most of us will agree with the view that disease is the inductive experiment of nature which the investigator must observe if he is to develop rational hypotheses to test by experiment.

Although personalities were not particularly noted in these articles it is evident that the qualified and alert clinical pathologist appears to occupy a happy position in the field of clinical and hospital investigation in that he is capable of viewing both the ascent and descent of the ladder from the top. If he has attained or can acquire the proper investigative or inquisitive mood he is in a position to intelligently foster this investigative attitude and promote public welfare in matters pertaining to preventing and combating human diseases.

<div align="right">H. J. CORPER.</div>

SOCIETY NEWS AND NOTICES

The Eleventh Annual Convention of the American Society of Clinical Pathologists will be held in New Orleans, Louisiana, May 6–9, 1932. Dr. F. M. Johns, Chairman of the Committee on Local Arrangements, has made arrangements with the Jung Hotel to serve as headquarters for the Society. The management of the hotel has agreed to allow members of the Society to keep their reservations at the hotel throughout the meeting of the American Medical Association. Members are urged to make early reservations.

Although definite plans for the meeting have not been completed, Dr. Johns has already announced a most unusual treat. He has arranged with Dr. Denny for the Society to spend Sunday, May 8, at the National Leprosarium where a luncheon will be served and clinical demonstrations on leprosy will be given.

Dr. A. S. Giordano, Chairman of the Program and Exhibit Committee, is desirous of having titles for papers submitted to him as early as possible in order that the program may be properly prepared. No papers will be accepted for the program unless titles are submitted before April 1, 1932.

Members desiring to compete for the Ward Burdick Award must submit summaries and outlines of their papers to Dr. A. G. Foord, Pasadena Hospital, Pasadena, Calif.

The Treasurer of the Society wishes to thank the members for their prompt response to his request for the payment of dues. Names of members, whose dues are unpaid by January 10, will not be included in the mailing list of the JOURNAL.

Members of the Society are requested to notify the Secretary promptly concerning the death of any member of the Society, that the name might be passed on to the Committee on Necrology.

Dr. Dudley A. Robnett, Associate Professor of Pathology at the University of Missouri, resigned, effective September 1, after nine years as a member of the Department to accept the position of Associate Professor of Surgery of the University.

Dr. Clarence C. Pflaum, formerly Instructor in Pathology of the University of Minnesota School of Medicine, has been elected to the position of Assistant Professor of Pathology at the University of Missouri School of Medicine.

Dr. Warren T. Vaughan has recently returned from several months travel in Europe.

The Editor of the JOURNAL will represent the Society at the meeting of the Council of the Union of American Biological Societies at New Orleans on December 28th.

From time to time a commercial firm selling laboratory supplies produces a commodity of unusual value. Occasionally the JOURNAL will call attention to some of these items. No one will deny the value of the contributions of the reputable supply houses to the advancement of laboratory sciences. Such a supply house is the Arthur H. Thomas Company which has always been willing to cooperate with clinical pathologists in the development of new apparatus and chemicals Recently they have brought out the most pretentious catalog ever issued in this field. It contains 1,044 pages listing 11,814 pieces of apparatus and 2,762 reagents. This catalog is a veritable encyclopedia for which clinical pathologists should be very grateful.

BOOK REVIEWS

The Significance of Water Borne Typhoid Fever Outbreaks 1920–1930. By ABEL WOLMAN AND A. E. GORMAN. Pp. x + 82, 1931, Baltimore, The Williams & Wilkins Company, $2.00.

Those public health officials who have been accustomed to extol the effectiveness of modern methods of preserving the nation's health, will read this excellent monograph with some alarm and even dismay. In it the authors review the history of water-borne typhoid fever outbreaks during the last decade. They give data on almost 300 such outbreaks and significantly point out the fact that over two-thirds of those in the United States and three-fourths in Canada were in cities of less than 5000. By far the greater amount of water-borne illness was due to defects in collection, treatment, storage or distribution of water. Forty per cent of these outbreaks were due to these defects rather than to pollution of the raw water at its source.

The evidence clearly points to gross errors in administrative attention, while inexcusable repetitions of outbreaks occurred in five cities and in one of these from the same cause. Unprotected cross-connections between polluted fire or auxiliary water supplies and public water systems were the most important single cause of water-borne outbreaks.

The authors give the legal actions taken against administrative officials and cities in damage suits brought as a result of typhoid epidemics, making an interesting and imposing array of incidences.

The book should be read, not only by health officials and those having to do with the water supplies of our cities and towns, but by citizens interested in the health of their communities.

Pathology, Bacteriology and Applied Immunology for Nurses. By ROBERT A. KILDUFFE. Pp. xiii + 324, 1931, New York,

Milwaukee and Chicago, The Bruce Publishing Company,
$2.50.

This book is intended to give the nurse an introduction to the
fields of pathology and bacteriology which although elementary
is a little more extensive than is usually offered in this type of
book.   The first part which is well illustrated and well organized
deals with pathology.   In this section however, there is a chapter
on animal parasites which needs to be rewritten to avoid a num-
ber of errors in names and facts.   At best the nomenclature in
this field is difficult but the misspelled and incorrect names that
occur in the text will be confusing to the student.   In the second
part dealing with bacteriology the author again encountered
difficulty with nomenclature, for example, in the chapter on
Special Pathology reference is made to *Clostridium tetani*, while
in the chapter on bacteriology one finds only the name *Bacillus
tetani*.   Nevertheless the chapter is adequate for the use of
nurses.   In the chapter on Applied Immunology the author dis-
cusses serums and vaccines and concludes the book with ex-
cellent chapters on the relation of the nurse to the education of
the public, in particular in relation to the propaganda of anti-
vivisectionists and finally concludes with a valuable chapter
dealing with methods for collecting specimens for the laboratory
and laboratory exercises.

A Text Book of General Bacteriology.   By EDWIN O. JORDAN.
    Pp. 819, 1931, Philadelphia and London, The W. B. Saunders
    Company, $6.00.

The tenth edition of this text book will meet with general favor
as have the last editions of this well known text.   The author
has evidently had a struggle with his conscience to permit him to
use the new generic names that are fast becoming popular in
bacteriology.   He has accepted almost all of them even though
reluctantly.   One can find, however, a few places in the next
where through an oversight the old names are still retained.   One
may question the inclusion in this type of book of the paragraph
on the sterilizing process in surgical operations.   Since it is not
adequately covered the information given might be quite mis-

leading. Although most of the text seems to have been rewritten, none of the new and excellent cultural methods for tubercle bacilli have been added since the last edition and one finds there no discussion of the use of animals for the diagnosis of tuberculosis.

One may seriously question in this day the advisability of including a chapter on protozoa in a text book of bacteriology. If the chapter is well done as it happens to be in this text, the author must call upon some specialist in the field to write the chapter for him. This is a tacit admission of the fact that the field lies outside of the domain of the bacteriologist and since we have perfectly adequate books dealing with this phase of the subject, it would seem that the time had come for eliminating such chapters from text books devoted to organisms that belong to the plant kingdom.

None of these objections, however, are serious enough to in any way affect either the high regard with which this book is held by students of bacteriology or its enviable position among scientific texts.

The Pathogenic Streptococci. By DAVID THOMSON AND ROBERT THOMSON. Annals of the Pickett-Thomson Research Laboratory, pp. vi + 441, 1931, London, Bailliere, Tindall and Cox, and Baltimore, The Williams and Wilkins Company, $10.00.

This volume includes a monograph on the rôle of streptococci in erysipelas, another on an historical survey of researches on the rôle of streptococci in skin diseases and lastly one on the rôle of streptococci in measles. Serious students who are concerned with this group of organisms have come to await with eagerness the appearance of these monographs. They are unique in bringing together practically all of the available literature on a subject, critically analyzing it and drawing therefrom a set of conclusions. In the first monograph the authors conclude that erysipelas is caused by a hemolytic streptococcus. They conclude that the organism *Streptococcus erysipelas* (Fehleisen) is different from *Streptococcus pyogenes* basing their conclusions on laboratory chemical tests, serological tests, skin tests, passive

immunity skin tests, the blanching test and the bacteriophage test, together with certain clinical facts. The historical treatment of the rôle of streptococci in skin diseases is well done and points out the many errors that have been made by previous investigators and the many valuable contributions that have been offered. After a thorough treatment of the subject of the relation of streptococci to measles and citing their own experiments, the authors are inclined to the belief that the organism of measles has not yet been discovered but that experiments so far favor the view that it is caused by an invisible filter-passing virus which will not grow on any culture mediums suitable for ordinary bacteria. The authors review work on the prophylaxis of measles and its treatment with convalescent serum as well as epidemiological factors and the control of the disease.

RHINOSPORIDIUM SEEBERI IN NASAL POLYP*

The Fourth North American Case†

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We owe to Ashworth[1] the first satisfactory description of the organism known since his work as *Rhinosporidium seeberi*. The organism was first recorded by Seeber[6] from Argentina in 1900 and was regarded as a protozoon allied to coccidium. Independent discovery of the organism was made in India whence the first case report was presented by O'Kinealy.[5] As in Seeber's case, the parasite occurred in a nasal polyp and the lesion was interpreted as a coccidial infection or local psorospermiosis. Minchin and Fantham[4] studied O'Kinealy's material and concluded that the organism possessed affinities with the Neosporidia and with *the simpler Haplosporidia. They created for it the genus* Rhinosporidium and appended the specific name *kinealyi*. This classification and name was generally adopted until Ashworth demonstrated Seeber's priority and emended the name to its present form. As a result of prolonged and painstaking study Ashworth was able to point out highly significant morphological inaccuracies in the earlier studies. Upon the basis of his own findings he reinterpreted and clarified the developmental cycle within the human tissues and concluded that the organism belongs to the lower fungi rather than to the sporozoa. He placed it therefore among the Phycomycetes and, in the absence of demonstrated mycelium, in the suborder Chytridineae.

No detailed statement of previous observations nor of the complete morphology will be attempted in the present report.

At the best this could be no more than a condensation of the information contained in Ashworth's monograph while for the reader to whom that source is inaccessible the essential facts have been reviewed by Weller and Riker[7] in a recent report that is readily available to American readers. Since a brief summary may however be desirable in the interest of orientation it may be stated that recognized rhinosporidial infection is an uncommon lesion.

Some twenty-five or thirty cases have been reported from India and Ceylon. Three are known from Argentina, all noted by Seeber. An organism of apparently identical type was found in a nasal polyp removed from a horse in South Africa. At the time of Ashworth's publication (1922) only one case had been reported from North America. The most common site of infection is the nasal or the nasopharyngeal mucous membrane, but in occasional cases the lesion has occurred in the conjunctiva or the lachyrmal sac, the uvula, in one case in the external ear and in one upon the glans penis. Most of the subjects have been young persons. Thus of twenty-two cases in which the age is known, fourteen patients were less than twenty years of age, four were twenty-one to twenty-six years and four were older. The youngest patient was eight years old, the oldest fifty-five years. No case has yet been recorded in a female. Within the lesion the organism is found in great numbers and in all stages of development. The inflammatory tissue response results in polyp formation. Single or multiple lesions occur. The polyps grow slowly but in the nose may eventually produce marked obstruction, blocking the nasal passages and even projecting at the anterior nares or behind the uvula. In Seeber's case the tumor tissue removed weighed 20 grams. There is decided tendency toward recurrence after removal. Ashworth was able to follow his subject over a period of about four years during which time tumor tissue was removed on six occasions. The patient had already undergone operation six times during a preceding period of five or six years. Artificial culture has been attempted by several observers but with a single doubtful exception no growth has been obtained. Inoculation into a considerable

variety of animals including the monkey has likewise been without result.

The first case of this disease in North America was reported by Jonathan Wright[8] of New York. The subject was a farmer twenty years of age who had lived all his life near Memphis, Tennessee. He had undergone removal of recurrent and multiple nasal polyps several times over a period of five years. Sections of the tumor were submitted to Wright during the same year in which O'Kinealy was reporting his Indian case. On the basis of the early English descriptions, Wright identified the organism and reported his case four years later.

Lincoln and Gardner[3] reported the second American case in 1929. The subject was a forty year old man who had never been outside the United States and who had lived most of his life in the upper Mississippi valley region although there had been short periods of residence in Oklahoma and in Florida. His occupation is not stated. He had suffered a nasal discharge with occasional bleeding for about eight years when the nasal polyp was removed and the organism discovered in it.

The third case was reported by Weller and Riker.[7] The lesion was again a nasal polyp. The patient was a young man twenty-six years old, a native of Missouri. He had lived on a farm from the age of six to that of eighteen years and had never been outside the United States except for three days spent in Canada. The parents and five brothers and two sisters were living and well and none had suffered a similar condition. The nasal lesion was first noticed in 1926 shortly after the patient had received a nasal injury. A polyp was removed and there was no further complaint until after a second injury received early in 1928 when progressive obstruction developed in the nose on the side previously operated upon. A second operation was performed in May, 1929 and in the following month a third operation resulted in the removal of the polyp upon which the report was based.

THE PRESENT CASE

The patient was a negro boy twelve years of age. He was born on a farm in Georgia. When he was four years of age the family moved to New Castle, a

small coal mining village just outside Birmingham, Alabama, where the father has since worked as a miner. The boy had never been away from home except for a period of about six weeks spent on a farm in Montgomery, Alabama during the summer of 1922. He had an older and one younger brother and two sisters, all living and free of complaints, as were the parents. On October, 1929 the boy complained of an increasing nasal obstruction and was taken to the local physician, Dr. E. C. Payne, who referred him to the Norwood Clinic in Birmingham where Dr. H. S. Gherken removed a small polyp from the upper anterior surface of the cartilaginous septum on the right side just within the naris. The tumor was sent to the laboratory as a matter of routine and there received no particular attention until routine sections had come through and the organism was recognized. In July, 1930 the patient was seen and a recurrent polyp was found to be present at the previous site. It had excited no complaint and close questioning failed to reveal its probable duration. The patient did admit considerable mucous discharge from the affected side with occasional traces of blood. The polyp was now 1 cm. in diameter with broad base and dull red faintly lobulated surface scattered over which could be seen an occasional barely visible yellowish white dot.

The polyp was again removed with the snare and the area of attachment thoroughly cauterized. Before operation the nasal discharge was planted upon a variety of media including Saboraud's agar but the organism was not

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FIG. 1. SPORES LYING FREE IN THE NASAL DISCHARGE

Their presence should render possible a pre-operative diagnosis of rhinosporidial infection. Dilute carbol-fuchsin stain. × 690.

FIG. 2. A TROPHIC FORM IN A MIDDLE STAGE OF DEVELOPMENT

A well defined pore is present in the plane of section. It is directed toward the epithelial surface. Eosin-methylene blue stain. × 160.

FIG. 3. RIPE SPORANGIUM DISCHARGING ITS SPORES INTO NASAL CAVITY

Note how the epithelium has disappeared over the bulging outer pole of the cyst. Eosin-methylene blue stain. × 160.

FIG. 4. A MATURED SPORANGIUM DISCHARGING ITS SPORES INTO THE SUBEPITHELIAL TISSUE

Early abscess formation. The space above the open pore contains free spores and leukocytes. Iron hematoxylin stain. × 80.

FIG. 5. SPORES LYING WITHIN A MATURED SPORANGIUM

A small sector of the marginal zone of undeveloped spores can be seen at the lower left hand margin. The section was stained by the eosin-methylene blue method and shows in each spore a sharply differentiated blue nucleus and red "spherules." This differentiation cannot be brought out in the black and white reproduction as sharply as in the original. × 970.
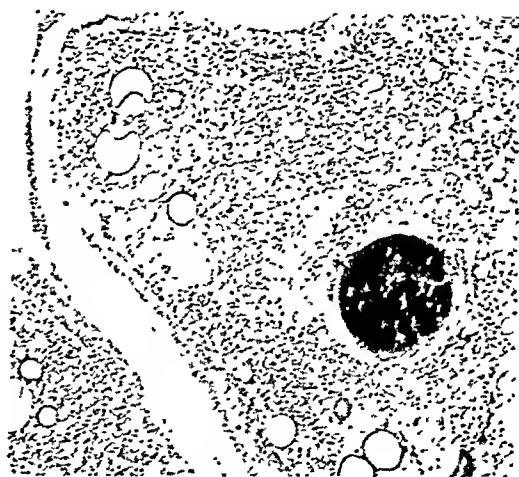
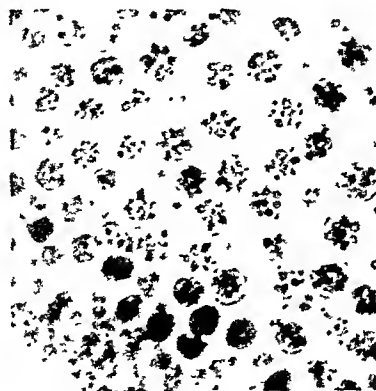FIG. 1

FIG. 2

FIG. 3

FIG. 4

FIG. 5

spores that are found in the nasal discharge as above noted and they may be seen in great numbers in sections lying upon the epithelial surface particularly in its crypt-like folds.

DISCUSSION

No other phases than those here sketched have been observed. As far as is now known they comprise the entire cycle of development within the human host. It is probable that the indicated cycle is continuously repeated through transformation of the spore into the encysted phase, but it is not known exactly how this takes place. Neither is it known how original infection occurs, nor how auto-inoculation at new sites is to be explained. From analogy with the known cycle in the sprozoa, the earlier students searched for an intracellular sporozoite development. They misinterpreted the spore structure and considered all its contained bodies as sporozoites or as a cluster of spores contained within a spore morula. Ashworth corrected these views and it was his great contribution to demonstrate that the so-called spore morula is a unit rather than a collection of equipotential spores. He believed that the spore develops directly into the trophic form, an opinion with which I agree. Just where or how this occurs is still a problem.

The question is bound up with that as to where the discharge of the spores normally occurs. According to descriptions which have been available, it appears to be assumed that the sporangia mature normally within the subepithelial tissues and discharge their contained spores directly into the stroma of the polyp. In our preparations this has seemed to be an accidental rather than a normal event. Most of the matured sporangia are found at the surface, where they are so situated that they can discharge their contained spores into the nasal cavity. Often they lie free within spaces outlined by infoldings of the surface epithelium and successive stages can be traced in the extrusion of the entire body through the covering epithelium from the site of maturation in the upper stroma. Ashworth describes such matured sporangia as occurring in the nasal discharge. Spontaneous discharge of spores could be seen in some cases, and unopened forms could

be made to rupture and discharge by being placed in water. The observation is of interest in connection with the suggestion from Indian observers that the infection may be water-borne.

After the spores are discharged upon the surface reinfection would require penetration of the epithelium. Such an event must be rare. The spore is a body distinctly larger than a red cell. There is no evidence that it is ameboid, indeed its structures suggest rather that it is a rigid body. Search of the present material supports the conclusion of others that intra-epithelial forms are extremely rare. We have found two spores lying within the lower zone above the basal line of nuclei. One early trophic form 8 by 9 micra in diameter is undoubtedly embedded at the middle level and a larger form 37 by 40 micra in diameter is probably intra-epithelial although open to some question of tangential sectioning. As compared with the great abundance of all stages of the parasite in the subepithelial tissue, these bodies are probably to be regarded as accidential imprisonments and it is not believed that continuous reinfection takes place by way of the transepithelial route. In the original infection and in the occasional instances in which auto-inoculation has occurred at new sites, it is probably necessary that the organism find its way in through an open wound of the epithelial covering.

It is possible that reinoculation and the initiation of new cycles may take place as the result of leakage of discharging spores into the upper stroma as surface sporangia discharge. When the ripening sporangium enlarges and bulges upward, the overlying epithelium is thinned and may disappear over an area large enough to allow of the eventration of the entire body. Again it may open only in a narrow sector over the site of the pore. As the pore opens and spores are discharged, some may make their way beneath the unseated epithelium in the space between it and the cyst wall, whence they are carried into the tissue by lymph currents. Suggestion of such an event may be seen in the sections, but may of course be artefact. Or again, the spores may find lodgement in the stroma within the fresh wound resulting from the expulsion of a discharging cyst (see fig. 3).

Finally, new generations may develop directly from spores

liberated into the deeper tissue by the rupture of cysts located beneath the surface.   This has been the explanation of the writers beginning with Seeber and O'Kinealy and is perhaps the reason why the sub-surface rupture of the sporangium has been accepted, at least tacitly, as the normal method of discharge.   In the present study this sub-surface rupture has appeared the most interesting phenomenon exhibited but, as already stated, it is interpreted as an accidental occurrence as contrasted with a normal surface rupture and discharge of the sporangium.   When it occurs the spores, possibly accompanied by a little mucinous embedding material, escape directly into the stroma.   A microscopic abscess results, starting about the open pore (fig. 4).   Unless this abscess is close enough to the surface to open at once upon it, liquefaction extends entirely around the sporangium so that it lies free in a space crowded with neutrophiles and large mononucleated phagocytes.   Spores, many of them in various stages of disintegration, lie in the space and the phagocytes are filled with basic or acid staining particles.

Occasionally a distintegrating spore itself can be recognized within a large phagocyte.   Eventually the cyst wall spreads wide open and becomes looped or contorted as though it were an elastic structure that has been relieved of tension.   Vaughan, in describing O'Kinealy's[5] specimen remarked this suggestion of "a certain degree of elasticity."   Very often the convex surface of the distorted membrane is covered by a thick layer of immature spores, such as may be seen normally at the basal pole of the unruptured sporangium.   There is here a suggestion that the cyst has turned inside out.   In young cysts that have failed of development, or in those that have ruptured, the wall may fragment and particles may be found enclosed within giant-cells (fig. 3).   Giant-cells appear also about the abscess margins to assist in the removal of debris.   Resolution of the abscess is followed by contraction of the embedding tissue and the remaining central space is filled by the formation of a loose, often edematous, scar tissue containing a network of thick collagen fibrils, but relatively few active fibroblasts.   Within it may be found occasional early trophic forms of the parasite.   Such scar

areas in various stages of organization may be seen scattered everywhere through the stroma.

The cyst wall of the early trophic form is believed by Ashworth and others to be chitinoid. In later development there is laid down inside it a thick layer of material "indistinguishable from cellulose." The resulting stratification is often clearly defined in stained specimens. I have found that the chitinoid layer is stained intensely by elastic tissue stains so that it is sharply defined in the youngest forms down to diameters of 6 micra while in the adult stages it persists as a delicate outer layer and is still recognizable in the collapsed remnants lying within an abscess cavity. Orcein has been especially useful for this definition. The whole wall is stained intensely blue by Mallory's aniline blue connective tissue stain and this stain has also been useful in searching for the youngest trophic stages. The most generally useful stain has been eosin-methylene blue. It affords good differentiation of the finer details of the spore structure, the basic stained nucleus standing out in sharp contrast to the acid stained spherules (see fig. 5).

The old suspicion that reseeding of the tissue takes place from the spores liberated within the abscess cavity is believed to be well founded. Some of these spores probably resist the destructive activity of the hosts' cells and survive to be carried into the marginal tissue, or to develop within the scar tissue laid down at the abscess site. The adult spore, as it lies within the sporangium or upon the epithelial surfaces of the polyp, is outlined by a thin membrane or capsule that is sharply defined in eosin-methylene blue preparations and unstained, or at most merely indicated, by orcein or the connective tissue stain. In the trophic forms, from their earliest recognizable to their oldest stages, the latter two stains bring out with great sharpness the thick wall that is the main distinguishing feature of the encysted, as distinguished from the spore, stage. Within the abscess cavity, as occasionally within surface forms, there can be made out a thickening of the capsule and an increased density of its substance. As noted by Ashworth, this change is accompanied by a decrease in the size, and finally in the number of the spher-

ules. While no satisfactory series of forms has been traced, it appears probable that this "transformation" proceeds directly to the complete disappearance of the spherules and the acquisition by the capsular substance of orcein or aniline blue tingability with a median stage in which there is a well defined cytoplasm that becomes broken up into a dispersed granular state as the whole body becomes smaller and the trophic form is finally developed. These early trophic forms occur within recently developed scar areas and have probably developed here.

In view of the rarity of (recognized) cases of rhinosporidial infection, their wide geographical distribution is of extreme interest. There seems no reason to doubt that the organism reported from different parts of the world is a single form. Unrecognized cases have undoubtedly occurred but even with this possibility in mind it is still evident that successful inoculation of man must be characterized by a remarkable discontinuity. Our patient had always lived in a small village of a few hundred inhabitants where almost if not all disability comes under the observation of the physician who first recognized the present lesion. He recalls only one other nasal polyp among the population of the community. This was seen and removed ten years ago but the patient's subsequent history and present whereabouts are unknown. Except for this there has been no case suggesting a similar condition. None of the other members of the family show any evidence of infection nor does inquiry reveal any in relatives or playmates. The patient has never been in contact with the larger domestic animals except possibly during his six weeks visit to a farm eight years ago, when he was but five and one-half years of age.

SUMMARY

A case is reported of rhinosporidial infection in a nasal polyp. The patient was a twelve-year old negro boy born in Georgia and resident since the age of four in a small coal-mining village in Alabama. The source of the infection is unknown, no other similar condition having been observed in the community for at least ten years. The case illustrates the remarkable discon-

tinuity of the infection, of which more than forty known cases reported during the past thirty years have been observed in three widely separated localities, India, Argentina and the United States.

Preoperative diagnosis should be made without particular difficulty through recognition of the characteristic spores in the nasal secretions.

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GLYCOSURIA AND THE GLYCEMIC TOLERANCE CURVE

A Review

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The chief object of this review is to point out the practical application of the sugar tolerance curve as an aid in the differential diagnosis of glycosurias of uncertain nature arising in man. To this end a survey of the factors determining and modifying such a curve will be discussed as they have been obtained by various investigators throughout the world within the last twenty-five years.

Any discussion of glycemia obviously necessitates inquiry into two factors that regulate the concentration of blood sugar, the threshold on the one hand and the capacity of the tissues to remove sugar from the blood on the other. Claude Bernard, during the nineteenth century, brought the problem into clear relief. In 1913, Aage Jacobsen,[35,34] working in Faber's clinic, conducted a series of investigations on the sugar threshold. Others followed him, notably Faber, Karen Marie Hansen,[15,14,16,13] Hagedorn,[25] Hatlehol,[30] MacLean,[40] and Traugott.[51] The definition of threshold is that concentration of sugar in the blood beyond which it appears in the urine. Jacobsen fixed this at 160 to 180 mgm. per 100 cc. of blood for normal persons, using Bang's micro-method of determination. Higher or lower values are not infrequently found, Hatlehol finding figures as low as 116 and 126 mgm. per 100 cc. in normal persons. Faber and his school defined the properties of the threshold in the following manner: it is constant in every individual, independent of age and diet, the presence of diabetes or its duration. Its position differs, however, in different individuals. Faber thus conceived the threshold to

be an "inherent quality in the individual, a constitutional quality." It may change in the individual under certain conditions such as phloridzin administrations or pregnancy. Most investigators concur with this definition in so far as it applies to normal threshold. Traugott,[51] however, said that a renal threshold for dextrose cannot be determined, nor does he feel that the kidney has a certain point of permeability for dextrose. He contended that the so-called renal factor in glycosuria was only an apparent one, being the expression of processes taking place outside the kidney. Following the work of Lepine he felt that alimentary hyperglycemia in normal people is not caused by a passage of sugar through the gastro-intestinal tract by way of the liver into the blood, but is chiefly caused by the sugar elaborated within the liver in response to the alimentary stimulus. This sugar is not passed by the kidney since it probably circulates in the blood in a bound form. Very exceptionally sugar does pass from the intestine into the blood stream causing a glycosuria, but in that case the sugar value for plasma is significantly higher than that of whole blood whereas normally he found plasma and whole blood to contain the same sugar values. Thus he felt that he had explained, on extrarenal grounds, why certain cases of alimentary hyperglycemia show glycosuria and others do not as well as why the glycosuria, when it occurs, is independent of the height of the blood sugar.

This called to mind Benedict's glycuresis and suggested the interesting theory' on the nature of the glucose-threshold propounded by Folin and Berglund,[18] an excerpt from which is hereby quoted:

There can be no doubt as to the existence of some mechanism by which the excretion of glucose is normally absolutely prevented. If we here venture to advance something approaching a new explanation we realize that it may be wholly wrong, but we believe that it should be at least partly correct. Each of us independently has in former researches seen and emphasized the avidity with which tissues may absorb materials from the blood. And throughout this investigation we have agreed that it is absorption by the tissues rather than the glycogen formation which prevents excessive accumulations of the sugars in the blood. With reference to glucose we assume that the tissues always contain at least as high a concentration of free sugar as the blood plasma and probably more.

The glycogen formation need not begin until the tissues have begun to possess a much higher concentration than that present in fasting. Much absorbed sugar can thus be distributed without any large increases of the sugar in the blood. But the kidneys receive their quota of sugar, just as do the other tissues, and this increase of sugar does not involve the slightest degree of strain. The strain comes only when the holding capacity for free sugar is reached and when the glycogen formation must come into play to keep the sugar concentration within normal limits. The speed of glycogen formation is of a much lower order than is the earlier process of merely absorbing the sugar from the blood. At this stage, therefore, the sugar backs up in the blood and *the holding capacity of some tissues including the kidneys is exceeded.* As a result of the strain thus produced the kidneys are finally compelled to make use of a more efficient process than the glycogen formation for reducing the sugar concentration in the kidney cells and the elimination of sugar suddenly begins. That a real local strain has preceded the escape of the sugar is indicated by the fact that the sugar excretion once begun does not stop as soon as the blood sugar has fallen below the threshold, but, in fact, continues until the level of the blood sugar has gone away down, even to subfasting values (hypoglycemia). It is as if there had sprung a leak for sugar, a leak which cannot be immediately repaired. Yet the total amount of sugar eliminated need not be very large except in clinical or experimental diabetes, because other tissues continue to absorb sugar as well as to make glycogen.

We cannot here develop this interpretation in more detail because of its many ramifications. The essential point is that it makes clear why increasing concentrations of sugar in the blood below the threshold values involve no strain and no elimination of glucose. From our interpretation it also follows that excretion of glucose, as in emotional glycosuria and renal glycosuria, does not represent a finely adjusted normal process analogous to the excretion of sodium chloride or of waste products. It is also in harmony with the belief in the importance of not permitting diabetic patients to excrete any sugar at all, if it possibly can be prevented.

Hamman and Hirschman,[28] felt that there was displacement of the threshold in diabetes and nephritis, the latter usually having a high threshold. Faber felt that this was due to the method of threshold determinations and on the basis of the method developed by himself, A. Norgaard and Karen Marie Hansen, seemed justified in his conclusions. By determining cutaneous (capillary or precapillary) blood sugar values every five minutes after the ingestion of two dissimilar doses of glucose, amounts of 35 and 25 grams for example, the threshold value appeared between the two.

One of Faber's charts is herewith reproduced (fig. 1*) in which it is seen that whereas 25 grams of glucose caused no glycosuria, 35 grams did, though sugar appeared in the urine only when the glycemic curve had begun to fall from its peak. Obviously then, the threshold here lies between the peak of the two curves.

Normally, the threshold usually varies between 0.160 and 0.180 per cent though it may, even in diabetes, be as low as 0.116 per cent or as high as 0.240 per cent. Karen Marie Hansen held that in normal individuals there is no break over the threshold even if 200 to 400 grams of sugar are ingested but Malmros, showed that in persons
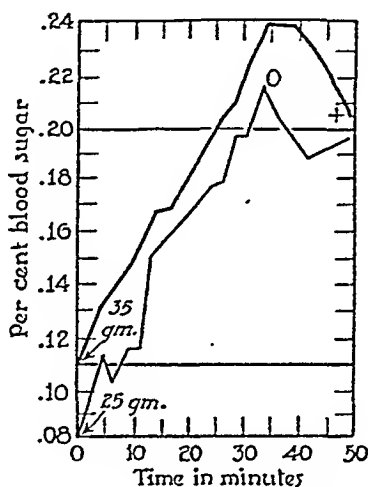

FIG. 1. REDRAWN FROM FABER

given 3 grams of glucose per kilogram there was a retention of 40 per cent of the ingested glucose in the stomach at the end of two hours. Thus the dictum that there is no assimilation limit for glucose in normal persons may not be altogether pertinent. The fact that sugar does not appear in the urine after its ingestion in large amounts, means that the body tissues present a mechanism of withdrawl that is extremely effective under ordinary circumstances. Karen Marie Hansen termed this exchange between tissues and capillaries the acceleration capacity of the organism,

* Except where otherwise stated the figures are constructed from data of investigators mentioned and are not reproductions.

a mechanism whose function is notably disturbed in true diabetes mellitus.

Since the position of the threshold determines the type of glycosuria and the course of the glycemic curve depends on the functional integrity of the removal mechanism, the best means of determining these factors becomes diagnostically important. On this account the choice of method is of fundamental importance, and choice of arterial (capillary) or venous blood sugar determinations arises. In this country most sugar determinations have been made on venous blood, whereas abroad and particularly
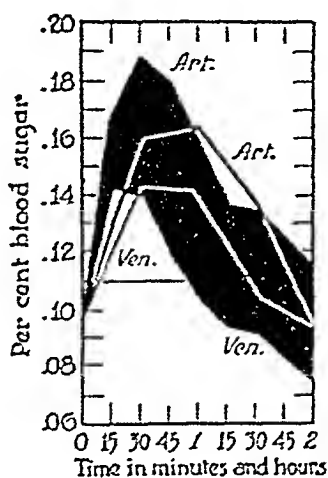

Fig. 2

in the Scandinavian countries, the use of arterial blood is more common, due perhaps to Bang's[3] introduction, of a practical micro-method for its determination. The arterial method should be the method of choice for the following reasons: first, the blood sugar percentage in the renal arteries must be the decisive factor in the occurrence of glycosuria; second, arteriovenous differences up to 50 mgm. are not unusual from thirty to sixty minutes after glucose ingestion (Friedenson et al.[23]), third, venous values are lower than arterial values in alimentary glycosuria, and finally, catching the peak of the rise often makes removal of blood at five minute intervals imperative.

Normals for arterial and venous blood have been established on

a series of individuals both by Foster,[21] and in Benedict's laboratory.[5] Figure 2 shows the two curves superimposed, Foster's values appearing in solid black.

All investigators feel that in normal fasting individuals, arterial and venous blood specimens give practically the same values. In sixteen normal cases Cori et al[9] found the difference to average 5.5 per cent; Dorle et al,[10, 11] found practically no difference in the two, either after fasting or after food intake in normals or in people with hypertension, although in lues they found the venous sugar after fasting higher than the arterial sugar. They also found that after fasting, mild exercise caused an increase of arterial over
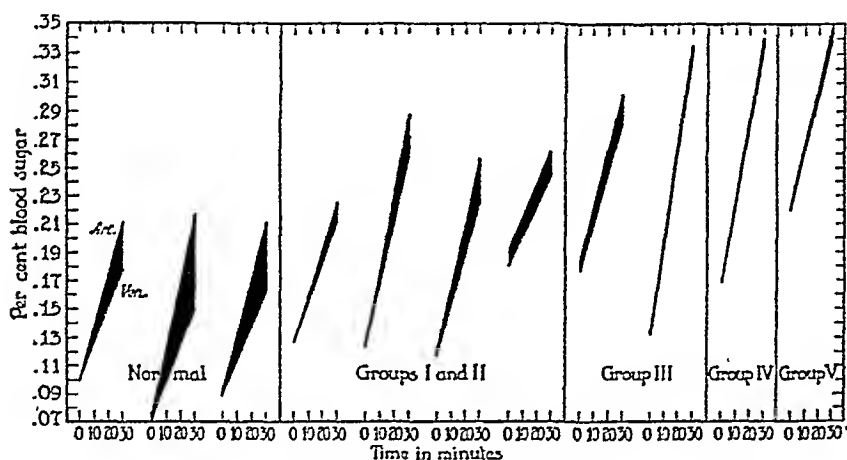

FIG. 3

venous blood sugar values. Practically all observers feel that the absorptive tissue mechanism accounts for the normal arteriovenous difference.

Investigations of the last few years point to the conclusion that, at least in diabetes, the presence or absence of arteriovenous difference is important. Figure 3 is constructed from figures of Rabinowitch,[44] who felt that the rate of utilization of glucose parallels the severity of the disease, which means that arteriovenous difference is greater, the greater the rate of utilization. By utilization he meant the combined mechanism of oxidation and storage. In figure 3 there is given first a group of normals.   Groups I and

II represent transient or postprandial glycosuria, probably non-diabetic; group III represents people with persistent glycosuria and fasting hyperglycemia.   In group IV are patients of diabetes who show acetone bodies in the urine but who get on well without using insulin.   Group V represents an exaggeration of group IV and needs insulin to meet daily requirements.   Roughly, two facts stand sharply defined as one observes the graphs, namely, that the fasting blood sugar tends to rise throughout and that the arteriovenous difference tends to disappear.
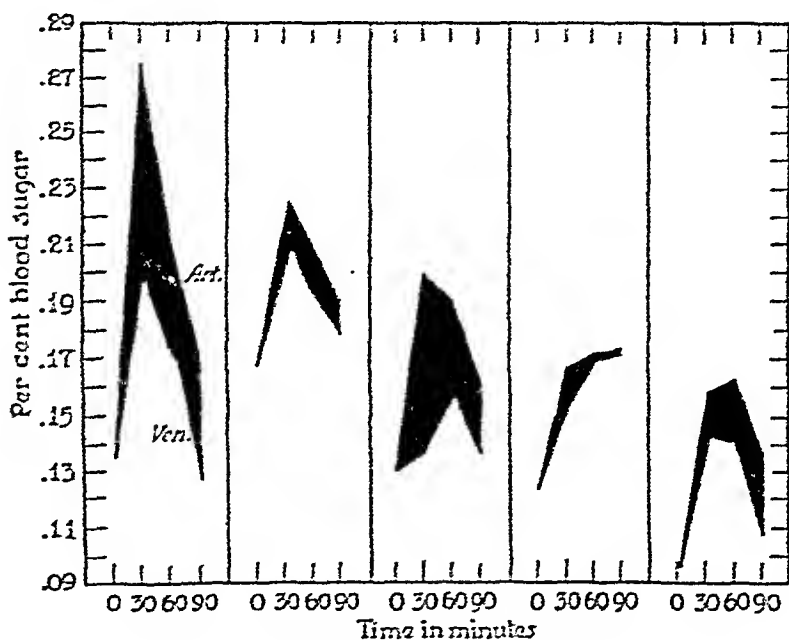
FIG. 4. REDRAWN FROM FRIEDENSON ET AL.

The importance of the arterial curve is emphasized in figure 4, reproduced from Friedenson, Rosenbaum and Thalheimer,[22] in which the lack of rise of the venous curve strongly emphasizes its unreliability as a method of tolerance investigation.   At the height of the curve one observes differences of 50 to 70 mgm. which serve to invalidate the venous method in the doubtful case.

The rest of this discussion is based on the sugar content of arterial blood, sugar here meaning reducing substance in the blood. Folin and Svedberg[19] concluded that the blood contains ferment-

TABLE 1
Normal Blood Sugar Values

AUTHOR	VALUES	MEAN	CASES	METHOD	REMARKS
	per cent	*per cent*			
Naunyn............	0.07 –0.10	0.080	4	Abeles	
Klemperer.........	0.08 –0.11				
Liefmann and Stern.	0.065–0.105	0.086	20	Knapp	
Weiland...........	0.077–0.101	0.090	6	Knapp	
Hollinger..........	0.065–0.100	0.090	10	Knapp	
Hollinger..........	0.068–0.089	0.080	5	Knapp	
Tachau............	0.064–0.088	0.083	16	Knapp-Tachau	Age: 16–54
Tachau............	0.061–0.084	0.078		Knapp-Tachau	Done on weight units
Frank and Bretschneider........	0.074–0.110	0.084	4	Bertrand	In plasma
Frank.............	0.08 –0.11	0.095	15	Bertrand	In plasma
Frank.............	0.098–0.102		3	Bertrand	In plasma. Age, 76
Rolly and Oppermann............	0.062–0.088	0.076	14	Bertrand	Fasting normal
Rolly and Oppermann............	0.078–0.107	0.096		Bertrand	In plasma
Grigault, Brodin and Buzand......	0.088–0.105	0.096	13	Bertrand	
S. P. Swart........	0.06 –0.11			Bertrand	
Kowarski..........	0.05 –0.11			Kowarski	
S. Strouse.........	0.04 –0.12	0.084	61	Kowarski-Strouse	
Schirokauer.......	0.072–0.120	0.103	25	Bertrand-Mockel-Frank	In serum
I. Bang............	0.10 –0.11		3	Bang (micro)	
Leire..............	0.06 –0.11	0.090	17	Bang (micro)	
Schumm and Hegler.	0.073–0.116	0.092	22	Bang	
Thannhauser and Pfitzner.........	0.07 –0.09	0.08	3	Bang	Normal
Bing and Windelor.	0.60 –0.113	0.096	16	Bang (micro)	
Ryser.............	0.063–0.105	0.086	21	Bang-Asher	Normal, fasting
Bing and Jacobsen..	0.06 –0.12	0.099	16	Bang (micro)	Normal, fasting
A. Jacobsen.......	0.083–0.128	0.101	14	Bang (micro)	Fasting
M. Elzas..........	0.080–0.115	0.100	25	Bang	Normal, fasting
H. Staub..........	0.073–0.113	0.096	55	Bang (micro)	Normal, fasting
Hollinger..........	0.076–0.100	90.01	5	Lehmann, Modifiz.	
Forschbach and Severin..........	0.06 –0.09			Forschbach and Severin	

TABLE 1—*Concluded*

AUTHOR	VALUES	MEAN	CASES	METHOD	REMARKS
	per cent	*per cent*			
W. Benningson.....	0.063–0.100	0.0875		Forschbach and Severin	Volume, per cent
W. Benningson.....	0.06 –0.096	0.0333		Forschbach and Severin	Weight, per cent
Levis and Benedict.	0.09 –0.11			Picrat-Color-imetrie	
A. O. Gettler and W. Bauer........	0.050–0.130	0.088	29	Picrat-Color-imetrie	3 hours after breakfast
Myers and Bailay..	0.09 –0.11			Picrat-Color-imetrie	
Salomon...........	0.09 –0.12		12	Picrat-Color-imetrie	Normal age, 17–18
Salomon...........	0.07 –0.12		20	Picrat-Color-imetrie	Normal and cured age, 20–48
Salomon...........	0.08 –0.15		10	Picrat-Color-imetrie	Weakness of age. No other signs
Wacker...........	0.12– 0.18			Wacker	
Watermann........		0.100	6	Wacker-Watermann	
Reicher and Stein..	0.09 –0.15			Reicher and Stein	
Taylor and Hulton..	0.05 –0.150				

able sugar other than glucose which is not maltose or any other di- or polysaccharide.

Of the arterial methods in use the chief ones are those of Bang, Hagedorn-Jensen,[26] Folin,[17] or Benedict,[5] together with numerous modifications of them all. With every one, it is important to hold rigidly by the chosen technique, being careful to eliminate all sources of error, and checking from time to time against solutions of a known sugar content. At the same time, as shown by Pickard and Pierce,[42] observed values should be checked against the biometric curves for error worked out by them. Absolute accuracy is imperative since it has been shown that with all of the methods the fasting value varies between 0.07 and .11 per cent and that observed values of more than 0.11 per cent are sug-

gestive of diabetes. Thus Liefmann and Stern,[39] Bang,[2] Staub,[45] Hagedorn,[25] Hansen,[29] Gray,[24] reported these figures as normal variants. Very occasionally values up to 0.115 to 0.12 per cent are found. Gray said in effect that the fasting value in 431 apparently healthy persons averaged 0.09 per cent. The unusual figures, however, of 0.12 per cent to 0.16 per cent were reached in as many as 7 per cent of these normals, thus leading to the alternatives: clinical judgment of normal metabolism is untrustworthy, or a considerable number of normals exhibit suspiciously large fasting figures. Punschel,[43] found fasting values in young people that averaged 0.094 per cent, using Bang's method. In individuals between fifty-eight and seventy years of age he found an average of 0.106 per cent, while the values in those between seventy and ninety-one years of age averaged 0.110 per cent, one of the latter reaching 0.133 per cent. Malmros,[41] however, never found fasting values above 0.11 per cent in people between fifty and seventy years of age. The practical unanimity of the fasting range is well illustrated by the tabulation collected by Punschel. Increased fasting sugar values, exclusive of diabetes, are found also in febrile diseases, especially malaria, in the latter weeks of typhoid and in severe cases of scarlet fever (Andresen and Schmidt.[1] Liefmann and Stern found hyperglycemia up to 0.28 per cent in lobar pneumonia without glycosuria. Williams and Humphreys[54] found a mild elevation of blood sugar from 0.12 per cent to 0.16 per cent in nine cases of carcinoma a marked increase in the last stages of nephritis and a moderate increase in cases of hypertension.

The technique of the tolerance test as employed on most of the curves here recorded has been worked out chiefly by the Scandinavian investigators and the modification presented by Malmros seems the most satisfactory. He gave the patient, twelve hours after the last meal, the equivalent of 1 gram per kilogram (of body weight) of glucose in a 10 per cent solution on an empty stomach. Arterial sugar determinations were then made at five or 10 minute intervals over a period of two or three hours. Hagedorn[25] earlier utilized this method and by means of it worked out what he termed the assimilation figure, which is the relation between the

amount of glucose administered and the amount that has circulated through the depots of the organism during the experiment in excess of the amount circulating before glucose was given. In normal individuals this figure is almost always above thirty. Malmros[41] found that he got the same curve with 0.5 gram per kilogram as with 1 gram, but not so high a rise when using 0.33 of a gram per kilogram. It is important to note that when he used 3 grams per kilogram of body weight he found 40 per cent of the ingested sugar present in the stomach at the end of two hours, yet the blood sugar curve fell to normal in two hours, which he interpreted as meaning that the withdrawal of sugar from the blood had been more rapid than the entry of sugar to the blood from the intestinal tract. Thus the course of the curve, in his opinion, depended chiefly on the withdrawal mechanism into which might enter the factor of absorption per unit of time. Therefore the higher curve obtained in diabetes on larger amounts of ingested glucose must depend on the withdrawal mechanism. The following quotation from Malmros[4] gives the exact technique of the procedure:

The experimental subjects had been on ordinary mixed diet for several weeks preceding the experiment. The determinations were made in the morning 12 hours after the previous meal. The person to be examined had been told to empty his bladder immediately on awakening in the morning. The bladder was emptied once more immediately before taking the glucose and once every half hour the next 2(–4) hours. Blood was taken fasting (immediately before the glucose) and afterwards once every 5–10 mins. during the first hour. The samples were generally taken most often (every 5 mins.) from 20–40 mins. after the glucose ingestion. During the 2nd hour the samples were taken as a rule every 15 mins. sometimes every 10 minutes. In a number of cases the examination lasted 3–4 hrs. In those cases samples were taken every half hour after the second hour. Duplicate samples were always taken, often 3 samples fasting. . . . . The dose of glucose ingested was 1 gm. per kgm. body weight; it was given as a 10% water solution. Merck's preparation, grape sugar, extra pure, anhydrous was used.

To avoid nausea the glucose solution has been cooled and flavoured with the juice of half a lemon. Each person took the sugar solution easily (in the course of 1–5 mins.), and none was nauseated. The time is counted from the moment when the experimental subject began to take the glucose solution. During the experiment the subject was sitting in a chair or lying in bed.

The frequent intervals, particularly during the first hour, are important because usually the height of the rise occurs between the first twenty and forty minutes.

Before continuing with a discussion of this type of curve, a brief reference to other types of diagnostic curves may not be out of place. Of the type obtained when the patient is given glucose
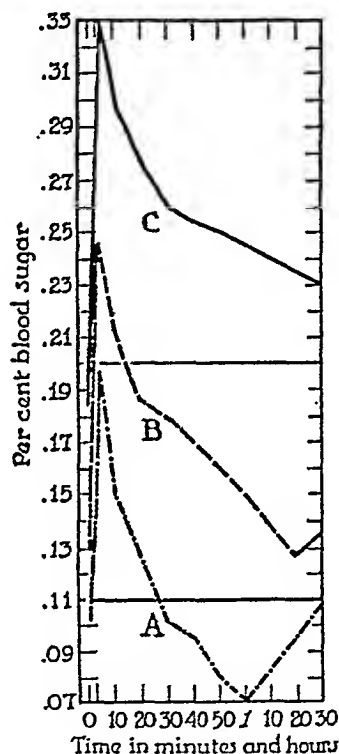

FIG. 5

by vein, one of the most satisfactory is perhaps that used and reported by Wislicki.[55] He gave 20 cc. of a 40 per cent dextrose solution by vein and the type of curve is reproduced from his data in figure 5. A, represents the normal, in which the fasting value is reached within thirty to forty-five minutes, B is the reaction of the mild diabetic and C that of the severe diabetic. The intravenous use of dextrose avoids alimentary sources of error and

possesses as well the advantage of rapidity and usability in cases in which the alimentary route cannot be employed.

The type of curve used by Seyderhelm[46] in von Noorden's clinic is termed the "Tages profil" of the blood sugar. This supplies a picture of the type of curve existing throughout the day and the technique is as follows: blood sugar test is taken before breakfast, again before lunch and finally before dinner, the patient fasting during the test. Normal individuals and diabetics receive as car-
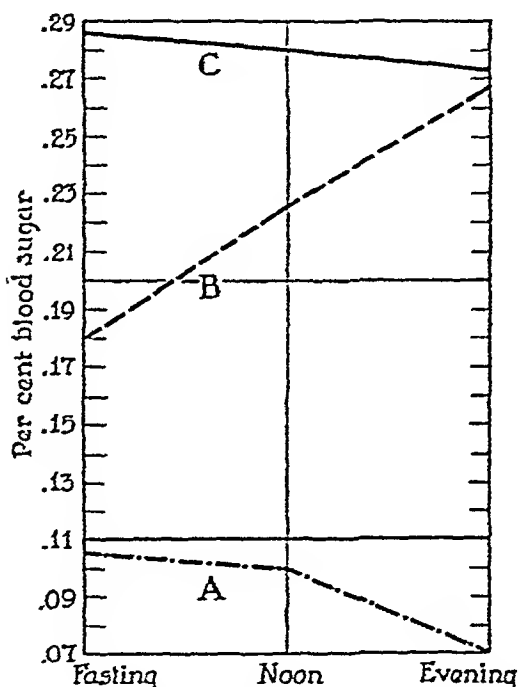

FIG. 6

bohydrate thirty grams of white bread on three occasions, first for supper on the night before, second for breakfast on the morning of the test and third for lunch on the day of observation. Repeated controls showed that in individual cases the carbohydrate portions could be varied, thus the morning dose could be given as two breakfasts of 15 grams each, but the second breakfast must be given two or three hours before the next blood sugar test. It was found, as shown in figure 6, that in normal individuals (curve A of the figure) the combined curve of the three blood sugar readings

depended upon the height of the blood sugar after fasting. Investigations showed that when the sugar value lies under 0.100 per cent the noon value often shows a slight rise. A marked fall of blood sugar value was never observed in the noon value of this group, while in the normal individual the evening value rarely rose above the fasting value. Curve C shows a graph from cases of diabetics in which insulin had not been given. In these the noon blood sugar was always lower than the blood sugar after fasting and continued study showed these to be cases in which insulin treatment was not necessary. Curve B is a graph from a group of cases in which the noon blood sugar showed a rise and in which insulin treatment very soon became a necessity. Many cases in this group also showed acidosis. From this procedure Seyderhelm concluded that a case with a noon fall of sugar has a good prognostic outlook. He further pointed out that the absolute height of the fasting value plays no rôle in the prognosis since this value depends on previous carbohydrate intake. Noon and evening values, however, seemed to him to indicate the ability of the pancreatic islands to form sufficient or insufficient amounts of insulin. If noon values sank particularly low the prognosis was always particularly favorable, these cases needing no insulin. A still greater evening fall in these cases was likewise a favorable sign. This type of "daily profil" of the blood sugar curve possibly has clinical application.

At this point one may also mention the diagnostic procedure employed by John.[36] He felt that if a blood sugar was taken twelve hours after the last meal, and if then a patient was given a heavy carbohydrate meal, it was only necessary to get a blood sugar determination exactly three hours afterwards, to settle whether or not the person was diabetic. If the blood sugar was above normal at this time the case was one of diabetes irrespective of whether or not sugar appeared in the urine.

Figure 7 shows the normal values obtained by Malmros[41] and Rabinowitch,[45] the former using the arterial method, and the latter using the venous method of blood sugar determination. The solid line represents Malmros' mean arterial value, the dotted one that of Rabinowitch. The greatest arterial variation (usually

within fifteen to thirty minutes) is up to 0.20 per cent and down to 0.11 per cent in two hours. Although these are Malmros' figures obtained on forty normal individuals most other investigators agree with them. Rabinowitch felt that the highest venous rise should not be more than 0.18 per cent, as indicated by the horizontal dotted line. The position of the arrow in the figure indicates the point, in Malmros' cases, at which the stomach is empty of glucose. In practically all of the figures the normal arterial limits, 0.20 per cent and 0.11 per cent (the latter the upper fasting limit), are shown by fine and heavy, unbroken lines respectively.
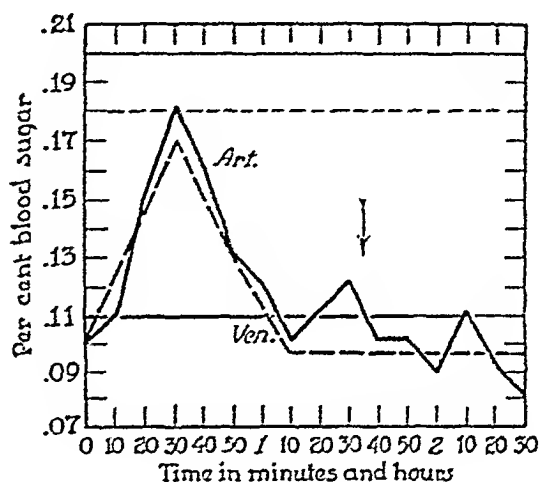

FIG. 7

Having determined the normal curve, the next question that arises is that of a classification of types of curves obtained in various types of glycosuria. By far the greatest amount of this work has been done by Scandinavians, particularly Jacobsen,[34] Faber,[15, 14, 16] Hagedorn,[25] Holst.[33] All these classified types of glycosuria on the basis of threshold and fasting Blood sugar values. Among various classifications that of Holst is chosen for reproduction. He recognized five types of glycosuria, based entirely on blood sugar examinations. There are two broad divisions, into the first of which fall cases of diabetes, defined by him as all those that show a fasting hyperglycemia, and into the second of which fall all cases of non-diabetic glycosuria. All of
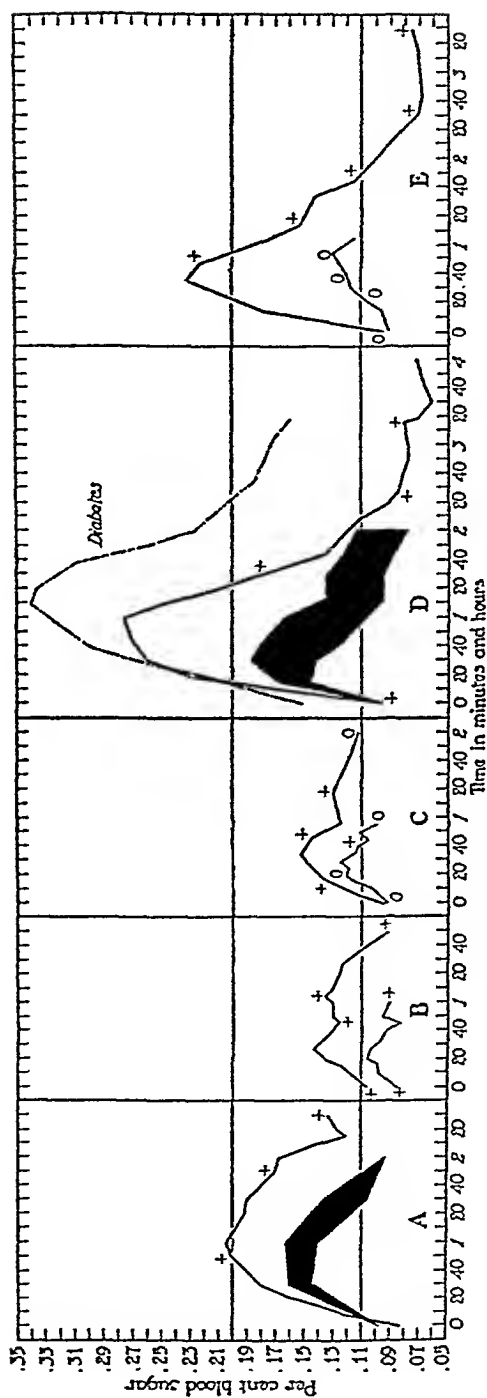
FIG. 8. MODIFIED AND REDRAWN FROM HOLST

the latter have normal fasting blood sugars without previous dietetic treatment.   Figure 8 illustrates his five types of glycosuria and is a reproduction of Holst's figures with modifications showing two normal curves and one diabetic curve for purposes of comparison.   Section A represents glycosuria with cyclic hyperglycemia. There is a normal fasting and threshold value, but the rise of the curve after glucose is greater than normal and there is glycosuria after meals.   The black graph is included for comparison and shows Friedenson's[22] normal venous and arterial values.   The plus sign means sugar in the urine at that point.   Section B represents renal diabetes.   The blood sugar after fasting is normal, the rise of the curve after glucose is normal but the threshold is lower than the blood sugar after fasting, resulting in a permanent glycosuria.   The fine line represents the values obtained after the ingestion of 5 grams of glucose, the heavy line that obtained after 63 grams of ingested glucose.   Section C represents cyclic renal glycosuria which differs from renal glycosuria only because the threshold is higher than the fasting sugar after fasting but lower than normal.   The upper limit for a low threshold in these cases is taken to be 0.14 per cent.   In this graph the fine line represents the curve obtained after 10 grams of ingested glucose and the heavy line that obtained after 50 grams.   The morning urine is free of sugar but glycosuria occurs after carbohydrate meals. Section D represents transition cases with permanent glycosuria. The blood sugar after fasting is normal; the threshold is lower than the sugar value after fasting, and after glucose an abnormally high rise occurs.   In this section Foster's normal venous and arterial values are represented in the black graph whereas the likewise interpolated dotted line illustrates a curve from a diabetic patient. Finally section E represents cyclic transition cases.   This differs from D only in that the threshold is higher than the blood sugar after fasting but below 0.14 per cent.   Thus the morning urine is sugar free but glycosuria occurs on feeding carbohydrates.   In renal glycosuria,as in transition cases the position of the threshold in certain cases can be such that the fasting blood sugar after fasting may be above or below it.   Thus from time to time a

threshold value of 0.09 per cent to 0.10 per cent will give positive or negative test for sugar in a morning urine specimen.

The present stage of knowledge concerning threshold is still comparatively a matter of dispute, thus the above classification has points of inadequacy. Since its formulation, indeed, other schemes of division have been put forward, but on the whole it still presents a definitely usable set of data. Recently Malmros[41] suggested another which with slight modifications is as follows:

1. *Glycosuria innocens with low threshold value and continuous glycosuria.* Fasting blood sugar normal, urine containing sugar the day round even on empty stomach. 18 cases. Tolerance test curve normal except in 4 cases where slight hyperglycemia remains after 2 hours.

2. *Glycosuria innocens, cyclic glycosuria.* Blood sugar curve of indefinite type. Fasting sugar normal. Tolerance curve generally normal. In two cases hyperglycemia being of longer duration that normal. 1 case up to .21%.

3. *Glycosuria innocens with pathological alimentary hyperglycemia.* Glycosuria of cyclic type, powerful hyperglycemia in two cases of longer duration than normal. Fasting blood sugar normal.

4. *Cases with history of glycosuria negative on exam.* Fasting sugar normal. Tolerance curve normal in 1 case rising to .21%.

5. *Positive reduction test during lactation.* Use phenylhydrazin test and fasting sugar to avoid diagnosing diabetes when it is lactosuria.

6. *Cases with subjective diabetic symptoms, but normal fasting blood sugar.* The material comprises 6 men and 2 women.

The fasting blood sugar was normal in all the cases. Larger amounts of sugar were in no cases excreted with the urine. The urine was sugar free in some cases, even after glucose ingestion. On the glucose tolerance test a normal blood sugar curve was found in most of the cases, in case no. 53 the hyperglycemia was rather stronger than normal and in cases nos. 47, 49 and 52 of longer duration than normal.

7. *Glycosuria of uncertain nature; glycosuria innocens or diabetes mellitus?*

Such classifications postulate blood sugar curves obtained under standard controlled experimental conditions. It cannot be too strongly emphasized that various factors modify the course of the blood sugar curve, so much so that unless these are ruled out, the curve may present entirely abnormal characteristics, simulating pathological conditions. An accurate knowledge of these modifying factors is therefore an absolute necessity if the blood sugar tolerance curve is to be used as a diagnostic aid.

THE EFFECT OF THE ABSORPTION RATE FROM THE INTESTINE

This has already been discussed and Malmros has shown rather conclusively that a dose of 1 gram of glucose per kilogram of body weight insures accuracy on this score.

EFFECT OF PRECEDING DIET

This, probably the most important of all the modifying factors, has received comparatively little attention up to the present. Bang[3] was perhaps the first to show that rabbits starved for sev-
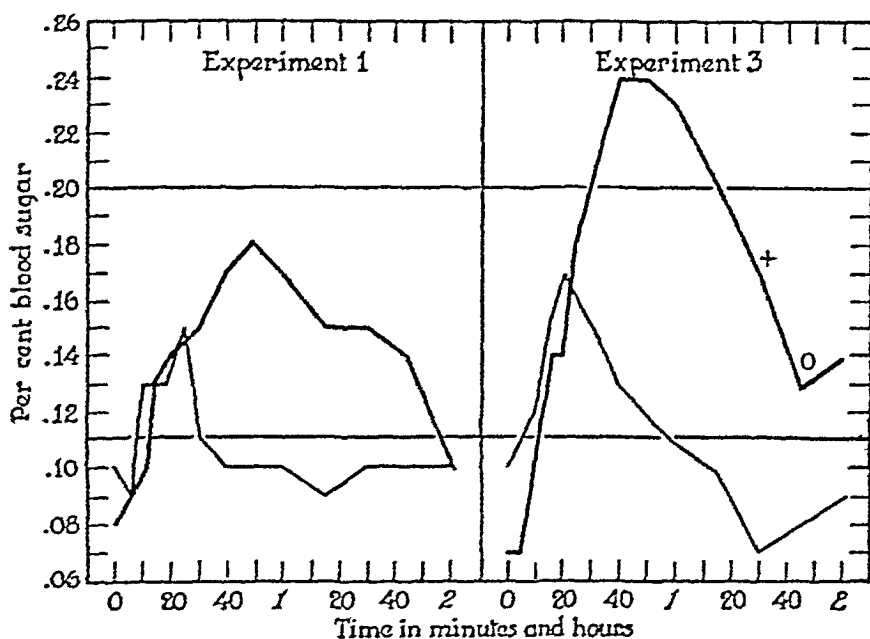
FIG. 9

eral days showed higher curves after glucose than fed rabbits. Traugott,[25] observed differences during the modified war diets, others likewise reporting altered curves with reference to diet. The following figures (fig. 9) show graphs constructed from some of Malmros'[41] data. In experiment 1 the fine line represents the tolerance curve obtained in the patient on a normal diet, while the heavy line represents the curve obtained after the same patient had been kept on a Petren diet for one day, the Petren diet being high in fat, low in carbohydrate and protein. In exper-

FIG. 10

FIG. 11

iment 3 of the same figure the fine line again represents the normal curve, while the heavy line is the result of four days on a Petren

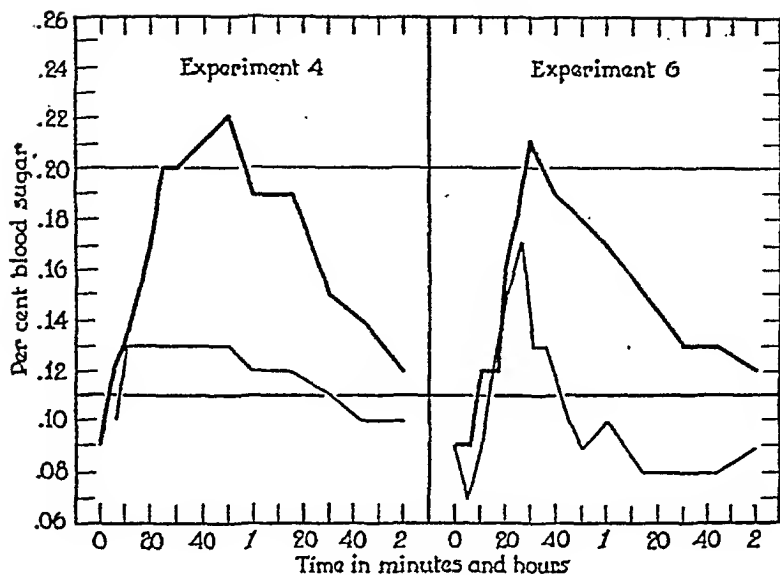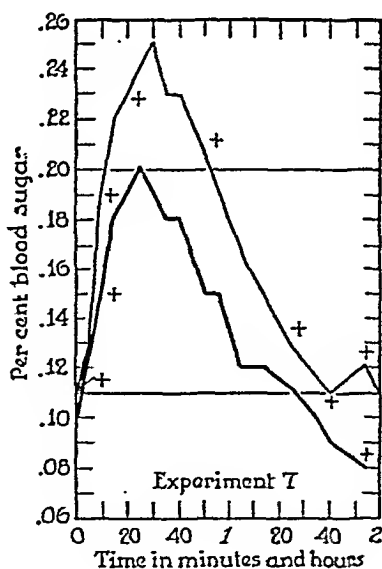diet.   Figure 10 represents the results of experiments 4 and 6, the heavy line in the former being obtained after a Petren diet of twenty-three days, the heavy line in the latter showing the modifications observed over a period of fifteen days.   In figure 11 the fine line shows the curve found in a patient who had been on a restricted carbohydrate diet for thirty-one years while the heavy line represents the curve that was found in the same patient after
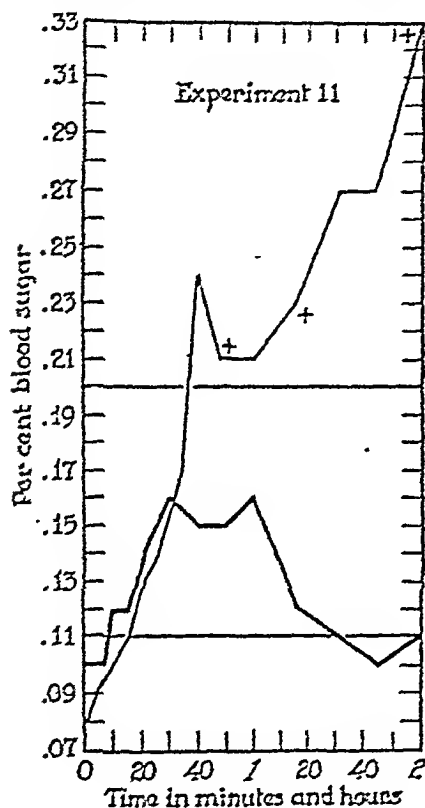
FIG. 12
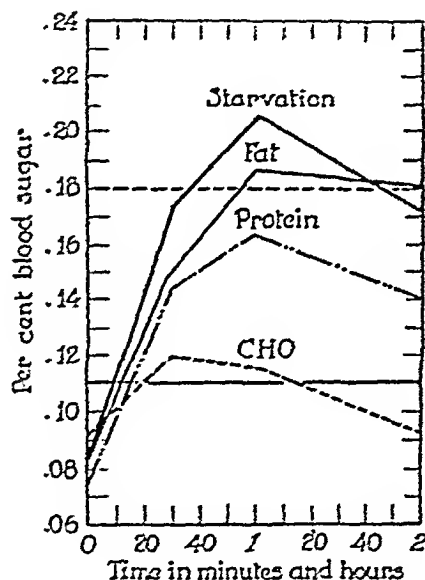
FIG. 13

two weeks on an ordinary restricted diet.   Figure 12 shows the results of experiment 11.   The fine line portrays the glucose tolerance curve after six days of fasting and the heavy line the curve in the same person obtained after ten days on an ordinary unrestricted diet.

In this country Sweeney[49] showed that the three major foods all affect the tolerance curve differently.   Figure 13 portrays Sweeney's results as modified by fats, carbohydrates, proteins and star-

vation, when the diet for two days previous to the test has been restricted to the foods named. These are venous and not arterial curves. Of the foods, fat shows the highest rise and carbohydrate the lowest, while starvation produces the effect observed in Malmros' excellent example. Together with Staub, Sweeney postulated the activation of an intermediate hormone which in turn stimulates the production of insulin. Thus when dextrose is taken a hormone is produced which stimulates the production of insulin and the result is only a moderate rise in blood sugar, whereas in diabetes, although the hormonic response is normal after the ingestion of dextrose, the amount of insulin that is produced is insufficient to store the dextrose as glycogen resulting in hyperglycemia and prolongation of the curve. In the patients receiving fats the activation of the insulin stimulating hormone has been reduced, thus again resulting in hyperglycemia. The same thing is true, from his point of view, in starvation; such theoretical tenets are at present, of course, far beyond any observed data.

Various ideas have been advanced to explain changes due to diet. Thus Elias,[12] using rabbits, turtles and dogs, showed that relatively small amounts of acid have a mobilizing effect on the glycogen of the liver, and that adrenalin or the adrenal glands have no part in the resulting hyperglycemia or glycosuria. The action of the acid is directly on the liver cells, glycogen for the most part leaving the liver cells as intact glycogen under its influence. Haldane[27] showed that in acidosis produced by ammonium chloride coincident with normal administration of carbohydrates there is failure to store glucose but not to oxidize it. Barrenscheen[4] in a series of experiments conducted on rabbits and dogs demonstrated that under controlled experimental conditions dextrose and levulose formed glycogen directly in the liver, while this was not a direct process with maltose and galactose. He further showed that after extirpation of the pancreas perfusion of dog livers with the blood of normal animals failed to cause direct synthesis of dextrose into glycogen which is of interest in view of Cori's work. Staub asserted in the first place that the glucose fixation ability of the normal liver is

a very variable one, in which point of view he was upheld by Forsgren[20] and his associates, who said that the liver has rhythmically acting functions that are to a certain extent independent of the supply of nutrition, that is, that it possesses an assimilating and dissimilating stage with reference to glucose.

Staub further felt that the ingestion of carbohydrate had a direct stimulating effect on insulin production and that the hypoglycemic phase of the ordinary tolerance curve is due to its over-production. Thus a low carbohydrate diet means less need for insulin with resultant decrease in its production. If, at this point, large amounts of carbohydrates are suddenly fed, however, enough insulin is not immediately produced to take care of it. Most authors feel that marked hyperglycemia depends on decreased glycogen deposition in the liver as a consequence of insulin deficiency. It is well to remember that in diseases of the liver carbohydrate assimilation is lowered and that there is the possibility of a low carbohydrate diet injuring liver cells. But whatever may eventuate in the realm of theory the practical demonstration of the influence of various diets on the blood sugar tolerance curve is of the utmost importance from a diagnostic point of view. The lesson to be learned is this; see that the patient is on a normal mixed diet for at least ten days or two weeks previous to the tolerance test, see also, that the food has actually been eaten. A careful checking of this source of error will do much to aid reliable interpretation of results.

THE EFFECT OF HYDRATION AND DEHYDRATION ON THE CURVE

Sweeney,[49] showed that hydrated animals exhibited a thirty minute delay in their postprandial blood sugar rise, the interpretation being that of a transient hydremia.

THE EFFECT OF EMOTION AND PAIN

Cannon et al[7] showed that cats, excited by the sight and sound of dogs for as short a time as one-half an hour, exhibited glycosuria, whereas after adrenalectomy, when exposed to the dog three times as long, they failed to exhibit it. Folin and Berglund,[18] fully substantiated emotional glycosuria on human beings.

More recently, however, Foster,[24] and Malmros[41] covering the same ground, came to the conclusion that there is no such definite effect.

THE EFFECT OF AGE

Various investigators have shown that the rise in the curve of individuals between fifty and seventy years of age is more marked and of longer duration than in younger people. Malmros,[41] found peaks up to 0.24 per cent as normal in such people.

THE EFFECT OF EXERCISE

Figures 14 and 15 are from Staub.[47]   In figure 14 the fine line represents the curve obtained while the person is at rest, whereas
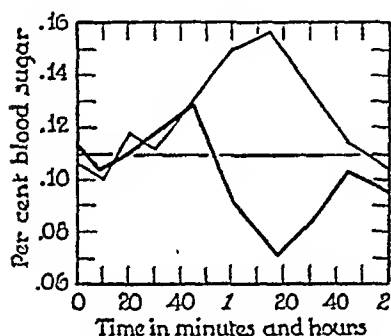
FIG. 14. REDRAWN FROM STAUB   FIG. 15. REDRAWN FROM STAUB
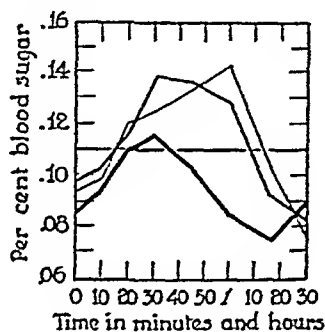
the heavy line shows the effect of hard work for one hundred minutes immediately following ingestion of 20 grams of glucose. In figure 15, the fine line again representing the resting value, the moderately heavy line shows conditions after moderate exercise whereas the heaviest line shows the curve after heavy work. Hoffmann[32] said that both the positive and negative peaks of the curve disappear during work in a well trained person, while Staub explained the hypoglycemic peak during work on the basis that while at heavy work the ingested sugar is immediately used and added glucose is brought in to compensate.

EFFECT OF DRUGS

Blotner and Murphy[6] felt that liver contains a blood sugar reducing substance active and nontoxic when taken by mouth, hav-

ing an effect on blood sugar concentration similar to that obtained by insulin. Koplowitz[35] found that in giving 0.4 gram creatin by mouth the blood sugar is above 0.200 per cent and there is some effect on the curve, especially after insulin. Cori's[8] theory on hyperglycemia following epinephrine is well known. There is an irregular effect on the tolerance curve following thyroxin, salyrgan, foreign protein injection and possibly following salicylates.

THE EFFECT OF DISEASE

According to Joslin,[37] v. Noorden and Isaac,[53] hepatic and pituitary disease, pregnancy, asthma, certain cases of arteriosclerosis, certain types of organic and functional nervous disease, hyperthyroidism, obesity, chronic arthritis and certain types of carcinoma may produce a pathological alimentary hyperglycemia. Cardiac edema (Taterka et al[50]), shows a low fasting sugar and a slower rise and fall of the curve than normal, while in eclampsia there is a marked hypoglycemia just before the attack.

HEREDITY

This factor has not been sufficiently recognized as influencing carbohydrate metabolism. A very careful study on this subject was contributed by Hjarne,[31] and the following quotation of his summary seems to have been borne out by the other available data. His term orthoglycemic glycosuria means renal glycosuria. The important fact to remember is that in so far as is known, no transition occurs between this condition and true diabetes mellitus. A quotation from Hjarne runs as follows:

Among 199 persons, interrelated by blood, the author has found:
orthoglycemic glycosuria in....................................... 18
glycosuria on glucose test in..................................... 6
glycosuria without diabetic symptoms in.......................... 13
diabetes mellitus in.. 7
transition case.. 1
glycosuria with BLS-curve of definite type in.................... 1
......

The cases of orthoglycemic glycosuria and glycosuria on glucose test and glycosuria without diabetic symptoms have been shown to be characterised by their clinical benignity and their non-development into diabetes mellitus.

These forms of glycosuria have been shown to be inheritable. They are inherited as monofactorial dominant characters.

Orthoglycemic glycosuria due to a lowered threshold, is to be regarded as a separate complaint.

No transition occurs between orthoglycemic glycosuria and diabetes mellitus. Diabetes mellitus has no effect on the hereditary transmission of orthoglycemic glycosuria. In marriage of persons who are probably both subjects of ortho-glycemic glycosuria no summation occurs in the direction towards diabetes; diabetes does not arise.

Orthoglycemic glycosuria and diabetes mellitus have probably a different origin. Their occurrence in the same families may be a chance coincidence due
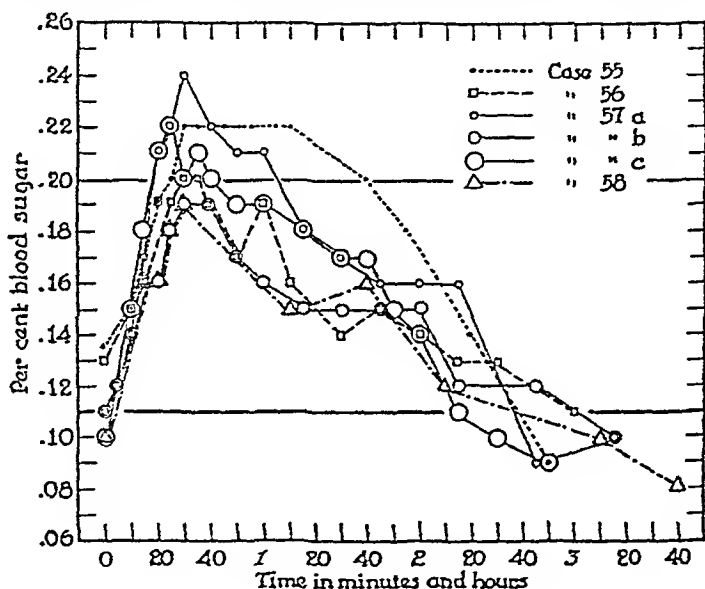
FIG. 16

to the fact that so far, it is mostly families with diabetes that have been sub-jected to careful search for glycosuria.

Figure 16 illustrates cases 55 to 58, inclusive, of Malmros,[41] series comprising six sisters and one brother all having glycosuria without subjective symptoms. He terms these "glycosuria innocens or diabetes mellitus?" There follows the quotation of his discussion,

Fasting blood sugar values of 0.13% were found in 3 of the patients (cases Nos. 55, 56, 57). [The figure shows lower fasting sugars for case 57, but his

statement refers to a sugar obtained previously—Author.]   In 2 of them (cases Nos. 58 and 60) the fasting blood sugar was normal on ordinary diet.   Nos. 59 and 61 were not more closely examined.   None of the patients have had any subjective diabetic symptoms.   Almén was pos. in 3 cases (Nos. 55, 56, 58) even on an empty stomach.

In case No. 55 a strong blood sugar rise (up to 0.22%) was found on the glucose tolerance test, after 2 hours the blood sugar was still high (0.18%).   As the patient has a too high fasting blood sugar value and the blood sugar curve on the tolerance test mostly resembles a diabetes curve, the diagnosis diabetes mellitus can appear certain.   There is, however, a possibility that a technical error can have been made on the blood sugar examinations, but the method has been controlled, as in all the other glucose tolerance tests, by control analysis on glucose solution of known concentration.

In case 56 the fasting blood sugar was also too high.   On the glucose tolerance test a blood sugar rise up to 0.20% was found, 2 hours later the blood sugar was still too high (0.14%).   This examination was made on the same day as the examination of case no. 55 but by another examiner.   The method was also controlled here.   Both these patients had kept diet, but the last few days before the examination they had been on ordinary mixed diet (but case No. 56 had not taken either sweet or sugar).   One could possibly surmise that the patients became hypersensitive for carbohydrate by the preceding dietary treatment and that the sudden change of diet could be the cause of the increased fasting blood sugar value and the marked hyperglycemia on the glucose tolerance test.   This does not appear very likely in the case of No. 55 as the preceding restriction of diet was only very moderate.   Fasting blood sugar of 0.13% was established on two different occasions in case No. 57.   On later examinations, however, the blood sugar was normal.   In this case the glucose tolerance test was made on three different occasions.   On two occasions the blood sugar rise was stronger than normal.   The hyperglycemia was of longer duration than normal in all three examinations.   One year elapsed between the second and third glucose tolerance tests.   During this time the patient had kept ordinary mixed diet but consumed no sugar or sweets.

In cases Nos. 58 and 60 mainly normal blood sugar curve was found on the glucose tolerance test, but the hyperglycemia lasted (case No. 58) somewhat longer than normally.

The observation time for case No. 56 is 18 years, No. 57 6 years, No. 58 8 years, No. 60 8 years.   Thus the glycosuria in these cases may be of a relatively benign nature.   The question then arises:—Are cases Nos. 55, 56 and 57 to be regarded as diabetes or not?   Case No. 58 ought to be designated as a glycosuria innocens with low threshold value ("renal" glycosuria).   In cases Nos. 55 and 56 also the threshold value may be low (pos. Almén on an empty stomach).   There could thus be found in the same family partly diabetes mellitus and partly benign glycosuria with a low threshold value.   It could even be said that cases Nos.

55 and 56 are an example that there can be a low threshold value in diabetes mellitus. If no consideration is taken of the blood sugar examinations but only to the mild course, it is almost tempting to classify all these cases as "benign glycosuria." Also it hardly seems possible that it is a question of 2 completely different diseases, which appear in this manner in the same family. At present this question cannot be decided. The continued course will furnish an answer to this. The cases are of exceedingly great interest, both practically and theoretically. It is just such cases as these that can answer the question whether glyco-
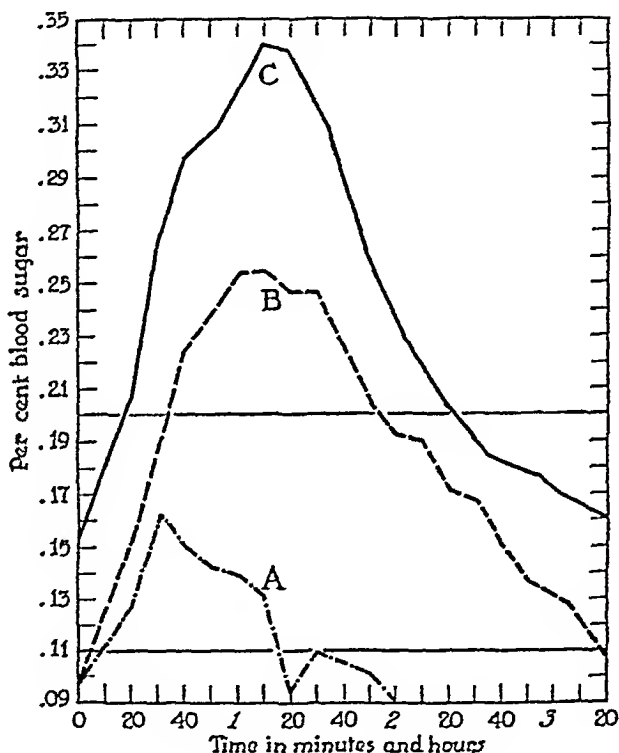
Fig. 17. Redrawn from Hatlehol

suria innocens with strong alimentary blood sugar rise is of a different nature than diabetes.

Figure 17 is taken from Hatlehol[30] case no. 53. A is the curve taken from the youngest daughter with cyclic renal glycosuria, B is the curve of the elder daughter who was a transition case and C is the curve of the father, who had benign diabetes. Under transition case is to be understood the type described by

Aage Jacobsen which is a glycosuria showing a great rise on administration of carbohydrates together with a low threshold. This, of course, corresponds with Holst's group IV shown in figure 8 under section D. Hatlehol, in common with others, found no progression into true diabetes in the transition cases.

Reference to figure 16 makes it obvious that in spite of the most accurate and refined methods there are still diagnostic problems. Two such will be illustrated from Malmros's series.   Figure 18
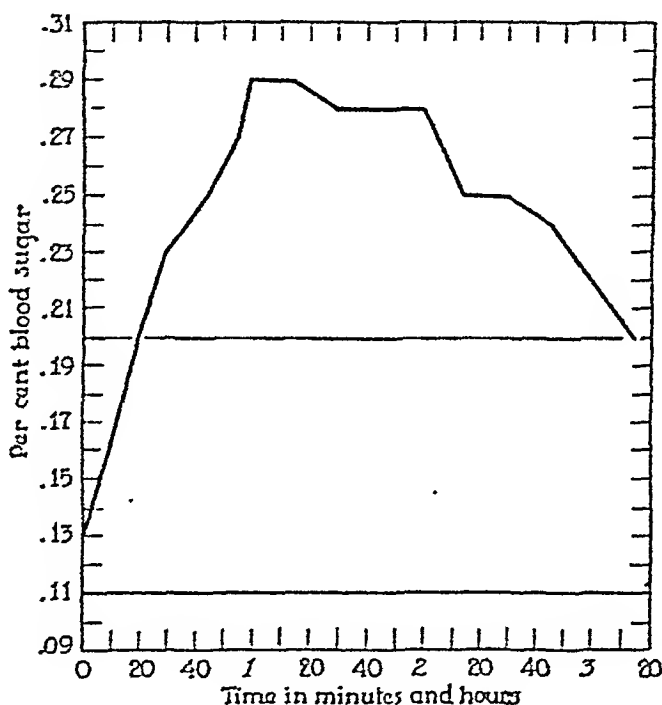
FIG. 18

shows the curve of case 62 in which the patient was under treatment for sciatica and whose history disclosed the fact that the father, mother and uncle had diabetes. Glycosuria was found and the first two blood sugar tests after fasting proved to be normal. From February 22 to March 9, on an ordinary mixed diet the sugars after fasting were not more than 0.11 per cent yet, as the figure shows, the tolerance test disclosed an abnormally high fasting sugar as well as a pathological curve throughout.

This case shows clearly that one cannot depend for diagnosis on a few normal blood sugar determinations after fasting. Figure 19 illustrates Malmros' case 64. There were no symptoms and the blood sugars after fasting were normal on ordinary mixed diet except on two occasions when they showed values of 0.14 and 0.13 per cent. The heavy line shows the first tolerance curve in which the fasting level is normal but the rise and duration are abnormal. The fine line shows the tolerance curve seven months later, which is entirely normal. The patient was on ordinary mixed diet dur-
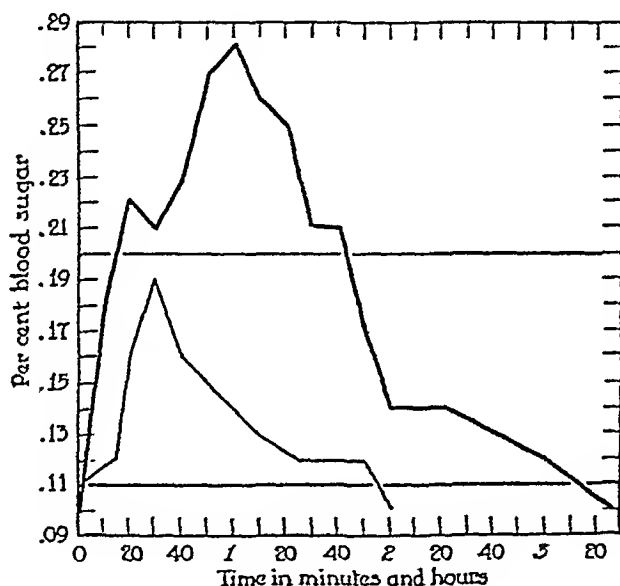
Fig. 19

ing the seven-month interval. To complicate matters still further, it was found that on the day following the second tolerance curve the fasting sugar was 0.13 per cent. Is this a case of diabetes or is it not?

DISCUSSION

It may be well to enquire briefly into the modifying factors that determine the shape of the blood sugar curve after the ingestion of glucose. There is the factor of rapid removal of sugar from the peripheral circulation during the period of absorption. Since the

early work of Claude Bernard the glycogen storing capacity of the liver has perhaps been foremost in our consideration and, therefore, the demonstration of a difference in sugar content between arterial and venous blood at the peak of the curve was at once unexpected and important. Among other things it partly explained the higher sugar readings obtained by Bang's method in contrast to the readings obtained by venipuncture. Folin, Trimble and Newman demonstrated that this rapid sugar removal from the periphery has less to do with glycogen formation in the muscles than with temporary storage without chemical alteration, particularly in the skin and subcutaneous tissue. This storing capacity is obviously diminished in diabetes, indeed in severe cases almost to a vanishing point. This might mean that the skin in diabetes carries a higher sugar concentration than in normal individuals, which lends new interest to the pathogenesis of various well known skin manifestations in this disease.

Physical removal of sugar from the circulation is not, however, the only factor at play, probably not even during the first minutes of the experiment, and in normal individuals it rapidly yields in importance to chemical removal, glycogen formation, and probably in a lesser extent to direct oxidation as well as synthesis of fat. The well known high respiratory quotients obtained after the ingestion of levulose are an indication in favor of the last alternative. The marked hypoglycemia following the hyperglycemia in normal individuals was discussed by MacLean and Wesselow as well as by Folin and Berglund before the arrival of insulin, and in the light of later experience must be taken as proof of a well working mechanism for the chemical disposal of sugar. Part of this mechanism must be the preparation or liberation of insulin, and it can be demonstrated, for example by the use of Woodyatt's pump for continuous injection, that time is required to raise it to a maximal working capacity. Using plenty of time for the stepping up of the sugar dosage, Berglund and Trimble reached decidedly higher tolerance levels than had been reported by Woodyatt and his collaborators, or than could be obtained by rapidly increasing the amount of sugar injected a minute. Therefore, no other single finding has done more to strengthen, theoreti-

cally as well as practically, the value of the tolerance curve than that of Malmros when he found 40 per cent of the ingested sugar still unabsorbed at a point when the blood sugar curve had already returned to normal after approximately two hours. Because of it one may well say that the declining part of the curve has little or nothing to do with the end of the absorption period but constitues a test for the mechanism of chemical sugar removal, or in most instances a test for the capacity to liberate insulin. This view harmonizes with the prolonged and even continuously rising curve in diabetes and constitutes the guide for the clinical use of the tolerance test. Theory and practical experience together make the prompt return of the sugar curve towards normal the most significant feature of this test when it is used for the study of sugar tolerance.

Up to the recent present it was believed that the metabolism of carbohydrate, protein and fat took place relatively independently of each other, at least as far as rate was concerned. No other dependence was suspected than the one discussed in terms of sugar and ketone body formation from protein, and of complete or incomplete oxydation of fat as a result of the simultaneous oxydation of glucose. The first well controlled experiments leading beyond this position were probably the ones carried out by Wilder and Boothby on the diabetic patient, Bessie B. It will be recalled that these experiments showed a very marked diminution of glucose tolerance when the protein level of the diet was raised without any simultaneous change in the total available glucose. A further step in the same direction is afforded by the recent work of Malmros and Sweeney with its highly significant demonstration of variability in the normal individual's reaction to glucose as the result of the characteristic dietary preceding the tolerance test. Thus when on a high fat diet or on prolonged fast, the individual responds to the sudden intake of glucose exhibiting a diabetic curve, in sharp contrast to the normal curve which is obtained when the preceding diet is dominated by carbohydrates, as a freely chosen diet usually is. Surprising also is the length of time that is required, according to Malmros, for a maximal adjustment from one type of diet to another. No doubt these

recent experiments will stimulate new work of a confirming or modifying character, but already the clear realization of the facts as they stand calls for greater caution in the carrying out and interpreting of glucose tolerance tests.

SUMMARY

A summmary of the more important diagnostic criteria to be considered in using the glucose tolerance test follows:

1. Never determine the blood sugar after fasting or do a glucose tolerance test on any patient until that patient has been on an ordinary mixed diet, unrestricted as to carbohydrate intake, for some days or weeks.

2. Use a reliable and accurate arterial method, and within the first hour take samples preferably at ten, twenty and thirty minutes, since the peak of the rise for the most part occurs at these periods.

3. Remember that the normal fasting blood sugar, both venous and arterial, should not exceed 0.11 per cent by any method used, except in people more than forty years of age or in those having febrile diseases, anemia, cerebral hemorrhage, or hyperthyroidism.

4. Determine the tolerance curve by using 1 gram of glucose per kilogram of body weight after a twelve hour fast, giving the glucose by mouth in a 10 per cent solution.

5. Keep in mind the fact that under these conditions in arterial blood the normal tolerance curve should not rise higher than 0.20 per cent and that the curve should fall to 0.11 per cent or below at the end of two hours.

6. Threshold determinations should be made in any case where the curve shows any abnormality in the presence of glycosuria, so that the type of glycosuria may be tentatively classified.

7. Remember that a fasting blood sugar of more than 0.11 per cent on any occasion is almost undoubtedly indicative of diabetes.

8. Do not be satisfied with a single fasting sugar. In a doubtful case do repeated fasting sugars over a period of months.

9. All cases showing a normal fasting sugar with a pronounced hyperglycemic curve of long duration should be suspected of diabetes and put under observation.

10. In abnormal cases of doubtful origin use the fermentation or phenylhydrazin test in the determination of type for the excreted sugar.

In addition one must (*a*) take a careful family history for possible glycosuria; (*b*) eliminate diseases, other than diabetes, that may cause disturbance of carbohydrate metabolism; (*c*) remember that long periods of observations, even up to forty years, seem to show that there is no transition in the same individual from non-diabetic glycosuria in any of the five classes discussed in this review to true diabetes mellitus, and (*d*) in cases without glycosuria, tolerance curves higher than normal do not necessarily indicate diabetes.

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MORPHOLOGIC AND CULTURAL STUDY OF STAPHY-LOCOCCI WITH SPECIAL REFERENCE TO SOURCE

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This study was made in an attempt to evaluate the various Gram-positive cocci which occur in cultures of blood particularly, and in clinical bacteriologic work generally. Finding and reporting a staphylococcus in a large majority of cases is not of value to the clinician, as there is no apparent connection with the history or clinical symptoms. There are exceptions to this rule, for example, staphylococcic bacteremia following local infections with staphylococci or the association of staphylococci with boils, carbuncles and osteomyelitis.

This suggests that there is a sharply defined and restricted group of organisms responsible for certain types of primary infectious processes, and also that there are many miscellaneous organisms which are often encountered and which must be considered extraneous. There seems to be no classification available which allows the clinical bacteriologist to separate one group from the other without the expenditure of considerable time. Even then doubts arise as to the relative importance of the various tests employed.

Considerable work has been done on the classification of the mass-forming Gram-positive cocci, and has resulted in the accumulation of a large amount of data. Few of these data are useful in clinical work on account of the time consuming procedures employed and the number of strains of cocci encountered which do not seem to fit into any existing classification. Winslow, Rothberg and Parsons[8] studied 180 strains of staphylococci, 104 of which were from pathologic material; the remainder were

isolated from the skin or from air, dust and water. These investigators proposed a classification based on production of pigment, fermentation of lactose, and liquefaction of gelatin. Six species of staphylococci were given.

Hine[2] studied ninety strains of staphylococci assembled without selection from the following sources: blood, boils, throats, sputum, lesions of osteomyelitis, urine and skin. These he divided into group 1 (pyogenic) which fermented mannite, and group 2 (epidermidis) which did not ferment mannite. A serologic study showed that there were three types in group 1 according to absorption of agglutins, and two types in group 2. Julianelle[6] concluded that hemolysis by staphylococci is related to proteolytic activity. The twenty-five strains studied by him were alike in fermentation of sugar. Certain irregularities were noted in the liquefaction of gelatin. By the absorption of agglutinin test there were three groups with two subgroups. Hudson[5] investigated the staphylococci of throats of normal persons and the throats of persons with colds. He found that mannite is often fermented by *Staphylococcus aureus*. Kligler and Kraus[7] worked with thirty-three strains of staphylococci isolated from such sources as furuncles, abscesses, mastitis, pyodermitis, osteomyelitis and eczema. Of the thirty-three strains, twenty-six were pigmented and seven were white; twenty-five of the twenty-six pigmented strains fermented lactose and mannite, and twenty-two liquefied gelatin. There was poor agreement as to cultural characters among the white strains. Agglutination tests indicated four groups.

The most extensive investigation of the staphylococci since the work of Winslow, Rothberg and Parsons, seem to have been made by Yoshioka[9] who studied 180 strains with regard to fermentation of sugar, action on milk and gelatin, production of hemolysin and staphylokinase, agglutination, and pathogenicity (mouse). The sources of the 180 strains were as follows: 127 from incised abscesses or in pure culture in open wounds; twenty from normal mucosa, skin and air, and thirty-three from sources such that pathogenicity could not be predicted. Using the 127 strains obtained from what he considered to be primary staphylococcic

infections as a criterion for judging the value of the various tests applied, Yoshioka found that fermentation of sugar was the most valuable means of separating the pathogenic from the non-pathogenic staphylococci. Galactose, mannose, trehalose and lactose were positive with the 127 strains, and arabinose, raffinose and salicin were negative. These sugars were considered the most useful for differentiation while others were not helpful. The author then compared other tests with fermentation of sugar, and found that production of hemolysin was almost as good, since it gave 150 positive reactions as compared to 152 reactions with sugars. Liquefaction of gelatin gave a higher number than fermentation of sugar whereas the other tests gave lower figures. The chief criticism to be made is that the finding of Gram-positive cocci in pure culture in an open wound is not proof that they are the primary cause of the wound. Nor is it logical to assume that cocci found in pure culture in postoperative infections are necessarily of the same type as those found in spontaneous infections.

Dudgeon and Simpson[1] collected fifty strains of *Staphylococcus aureus* and eighty strains of *Staphylococcus albus* from "acute and chronic infections in man." All of the strains of *Staphylococcus aureus* liquefied gelatin and fermented lactose, and forty-seven fermented mannite. The strains of *Staphylococcus albus* gave inconsistent results. Hemolysis was produced on blood agar by 68 per cent of the strains of *Staphylococcus aureus* and 56 per cent of the strains of *Staphylococcus albus*. The precipitin reaction did not serve to separate the yellow and white cocci. Hucker and Rettger[4] pointed out that ammonium salts may serve as the only source of nitrogen for the saprophytic micrococci when used in a medium of definite chemical composition, whereas the parasitic forms fail to grow in such a medium. Hucker[3] presented a key for the identification of cocci included in Bergey's Manual under the genera Gaffkya, Staphylococcus and Micrococcus. He listed nineteen species and made the primary separation on production of pigment. Other characters were reduction of nitrates, liquefaction of gelatin and utilization of monobasic ammonium phosphate as a source of nitrogen.

In the present work 150 strains of cocci of the staphylococcus-micrococcus group were studied. They were divided into three groups of fifty strains each, according to source, as follows: group 1, scrapings from normal skin, group 2, cultures of blood in cases in which only an occasional colony, or at most a very few colonies, appeared on the plates, or in which growth appeared in broth but not on plates, and group 3, material from pyogenic infections. Exceptions to the foregoing are that, in group 2 one culture was included which was obtained from a typical staphylococcic bacteremia, and in group 3, five strains were included which were of doubtful significance, as well as could be judged from the original cultures. Of these five strains, three were from cultures of blood, one was from a culture from a prostate gland, and one was from culture of spinal fluid. The 150 strains were tested for ability to ferment lactose, saccharose, mannite and raffinose, to liquefy gelatin, to produce hemolysis on blood-agar plates and to produce pigment. The morphology was studied by preparing gram stains from nutrient agar slant cultures twenty-four hours old. The first twenty strains of each group were tested for ability to grow on a synthetic medium with ammonium oxalate as the source of nitrogen. Positive growths were not observed, and it was therefore concluded that organisms from the sources chosen do not often fall within the saprophytic group mentioned by Hucker and Rettger. The strains in group 3 were from boils in eighteen instances, from osteomyelitis in five, from wounds in eleven, from blood in six (four cases) from infected finger in three, from infected foot in two; from empyema fluid in two, from duodenal drainage in one instance; from the prostate gland in one; and from the spinal fluid in one.

It will be seen from table 1 that groups 1 and 2 are comparable, and that any one sugar will not serve to distinguish true staphylococci from saprophytic cocci. Mannite is more useful than any of the other sugars used. In order to get a fairly accurate cultural differentiation, lactose, mannite and gelatin may be used, and a test made for hemolysis. Most pyogenic staphylococci ferment lactose and mannite, liquefy gelatin, and

produce hemolysis when streaked on blood-agar plates. Six strains from group 3 which should be typical pyogenic staphylococci according to source failed in some one of these tests, yet morphologically they were typical of the pyogenic group. In this same group forty-five strains produced yellow pigment, whereas, five were not pigmented. One of the white strains which was obtained from a boil gave all the cultural and morphologic characters of the pyogenic type, and one yellow strain which was obtained from a culture of blood, did not correspond either culturally or morphologically with the pyogenic type. The latter case was one of infection of the foot following a bruise

TABLE 1

SUMMARY OF RESULTS OBTAINED IN THE VARIOUS GROUPS

SOURCE		CULTURES	POSITIVE FOR LACTOSE	POSITIVE FOR SACCHAROSE	POSITIVE FOR MANNITE	POSITIVE FOR RAFFINOSE	POSITIVE FOR GELATIN	POSITIVE FOR HEMOLYSIS	TYPICAL MORPHOLOGY	YELLOW PIGMENT
Group 1	Skin	50	39	46	13	2	20	12	4	5
Group 2	Atypical cultures of blood	50	41	49	16	2	29	26	5*	7
Group 3	Pyogenic infection	50	44	50	44	0	44	45	45*	45
Selected from group 3	Boils and osteomyelitis	23	22	23	23	0	23	22	23	23

* Includes three cultures from one case.

and a typical staphylococcus was obtained from the wound. Thus it is seen that with two exceptions the strains in group 3 could be separated into typical staphylococci on one hand and micrococci on the other by production of pigment alone. The occasional failure of lactose to distinguish the pyogenic staphylococci from the micrococci is well illustrated in the following: Three cultures were obtained from the same case, one culture of blood antemortem, one culture of blood postmortem, and one culture taken from a furuncle. All three cultures failed to ferment lactose even after thirty days, but otherwise they were typical of the pyogenic group, culturally and morphologically. The case was one of diabetes and furunculosis.
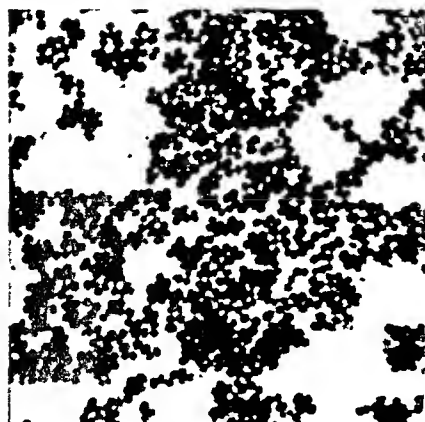
Fig. 1
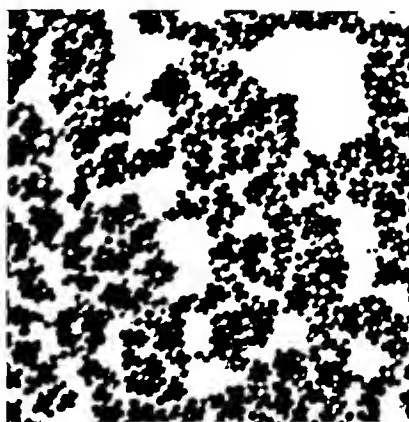
Fig. 2
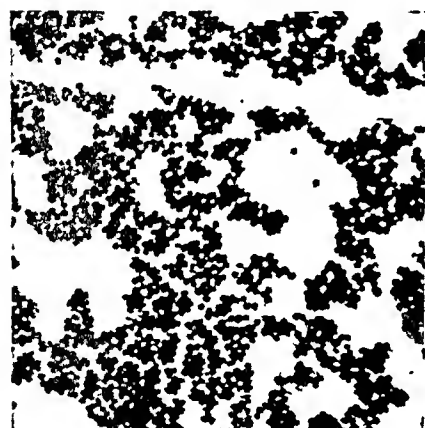
Fig. 3

Fig. 4
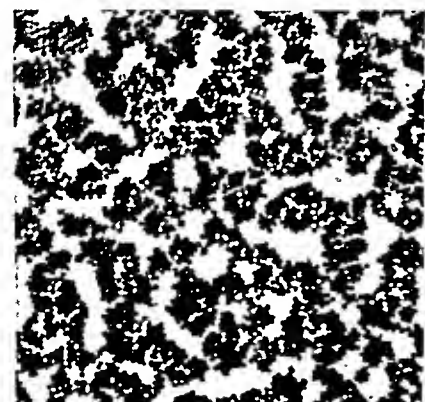
Fig. 5
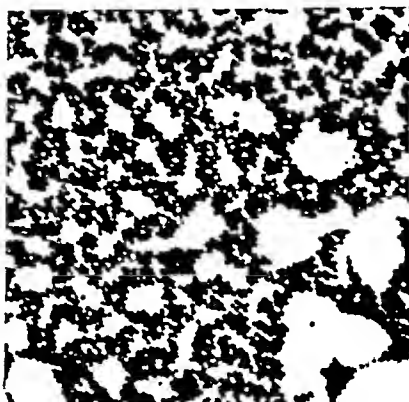
Fig. 6

Attention should be called to the similarity of the results obtained in groups 1 and 2. This suggests that the occasional colonies encountered in blood culture plates arose from organisms derived from the skin of patient or technician. In group 1 only four strains of typical staphylococci were found in a total of fifty, whereas in group 2, five strains of typical staphylococci were found in a total of fifty.

The greatest interest in this work is attached to the morphology. Little can be found in the literature or in textbooks

TABLE 2

GROUPING ACCORDING TO MORPHOLOGY OF MASS-FORMING COCCI ENCOUNTERED IN CLINICAL BACTERIOLOGY (PLATE)

FIGURE	SOURCE	GROUP	DESIGNATION	SIZE	LACTOSE	SACCHAROSE	MANNITE	RAFFINOSE	GELATIN	HEMOLYSIS
1	Skin	1	Micrococcus	1μ	+	+	−	−	−	−
2	Culture of the blood	1	Micrococcus	1μ	+	+	−	−	−	−
3	Skin	2	*Staphylococcus epidermidis*	0.7μ	+	+	−	−	+	−
4	Culture of the blood	2	*Staphylococcus epidermidis*	0.6–1.0μ	+	+	−	−	+	+
5	Osteomyelitis	3	Pyogenic staphylococcus	0.6μ	+	+	+	−	+	+
6	Furuncle	3	Pyogenic staphylococcus	0.6μ	+	+	+	−	+	+

which is helpful regarding the morphology of staphylococci and micrococci. Such descriptions do not mention the medium employed or the age of the culture, factors which are most important in determining the size and grouping of the cocci in question. The 150 cultures studied were all compared for morphology under as nearly similar conditions as possible. Gram stains were made from twenty-four hour cultures on nutrient agar slants having an initial reaction of pH 7.0. Care was taken to flame the slides before the smears were made so as to have the material spread evenly. Smears were made of such thickness

that fields might be observed which were well covered but not crowded so as to cause piling-up of the organisms unless they tended to do so naturally. The illustrations show what is considered about the right thickness of smears for easiest differentiation.

It was found that the 150 cultures could be divided into three types on the basis of morphology with the exception of two cultures from the skin which appeared to belong to the genus Sarcina. Figures 1 and 2 represent type 1 which comprises about 80 per cent of group 1, and 50 per cent of group 2. Most of the other organisms in these two groups fall into type 2, represented by figures 3 and 4. The typical pyogenic staphylococci in group 3 are represented by figures 5 and 6. Additional data concerning the strains represented by these figures are presented in table 2.

The organisms of group 1 were easily recognized by their greater size. The cocci appeared in clusters and tetrads. The organisms of group 2 were harder to distinguish from the typical staphylococci, which they more nearly approached in size. In general the organisms of group 2 were larger, less regularly round and varied more in size than those of the pyogenic type. The most helpful character in differentiation was the tendency to form clusters so that one organism lay above another on the slide. As opposed to groups 1 and 2, the cocci in group 3 were arranged in flat plates, almost all of them in one plane. Within these plates the cocci were so closely packed that they resembled the arrangement of cells in honeycomb. The outlines of individual cocci were distinct, owing to the distribution of the bacteria in one plane. These distinctions applied only to cultures examined under the conditions given, and not to smears made from broth cultures or from pus. A study of the morphology in hanging-drop cultures produced nothing helpful, chiefly on account of the difficulty of observing unstained bacteria.

COMMENT

On the basis of this study of 150 cultures it appears that most of the mass forming cocci encountered in clinical bacteriologic

work can be put into one of three groups. Those in group 1 appear to belong to the genus Micrococcus; those in group 2 correspond more nearly with the descriptions of *Staphylococcus epidermidis*. Both of these groups may well be looked on as incidental organisms in most cases, or as secondary invaders occasionally. Group 3 is essentially the pathogenic group. Some strains are white and correspond to descriptions of *Staphylococcus albus*, but most are pigmented and would be called *Staphylococcus aureus*. Most observers minimize the importance of production of the pigment as a differential character, since the pigment is variable under different conditions of culture, and since it may or may not be present even within the pyogenic group. The trinomial system formerly used designated these organisms as Staphylococcus pyogenes aureus, and Staphyloccus pyogenes albus. It would be more practical to combine both under the term *Staphylococcus pyogenes*. Strains fermenting raffinose were rarely encountered. Two were obtained from skin, two from cultures of blood, and none was obtained from typical staphylococcus infections. Three of these cultures resembled group 1 morphologically. The fourth resembled group 3 morphologically, it appeared as a single surface colony, on one of three plates used in making a culture of blood. Thus there is little evidence to justify a separate species within the pyogenic group corresponding to the descriptions of *Staphylococcus citreus*.

It is believed that a careful morphologic study of the mass-forming cocci obtained in clinical work will do much toward correlating the bacteriologic data with the clinical diagnosis. This can be done in twenty-four hours and has apparently given even more reliable results than the combined cultural tests including fermentation of sugars, and action on gelatin and on blood, which may require from one to three weeks.

SUMMARY

One hundred fifty strains of mass-forming cocci were studied culturally and morphologically as follows: fifty strains of cutaneous origin, fifty from atypical cultures of blood, and fifty from cases of pyogenic infection.

On morphologic grounds these 150 strains can be divided quite definitely into three groups, but less definitely by cultural procedures. The three groups are designated as Micrococcus, *Staphylococcus epidermidis* and *Staphylococcus pyogenes.*

It is believed that the morphologic study will offer a quick method of evaluating the various mass-forming cocci encountered in clinical bacteriology, and that it will greatly reduce the number of organisms reported as staphylococcus and increase the significance of those which are so reported, because of better correlation with clinical observations.

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MODIFICATIONS OF DIFFERENTIAL STAINS WITH SPECIAL REFERENCE TO THE TRICHROMIC STAIN OF CAJAL

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Many pathologists use the silver impregnation methods of Cajal and his pupils for the investigation of the nervous system, but very few know and practice the triple stain, primarily described by Ramon y Cajal[3,4] in 1897, modified later by Calleja,[1] and Gallego.[2]

Without doubt this method is one of the best for staining all kinds of specimens composed of different structural elements, and especially for the study of tumors. Briefly the stain described by Cajal is as follow:

(1) Place sections for five or ten minutes in a saturated or very concentrated solution of basic fuchsin.

(2) Wash rapidly in water to remove the excess stain.

(3) Stain for five or ten minutes in a solution of picro-indigocarmin. (Saturated aqueous solution of picric acid, 100 cc. indigocarmin, 0.25 grams).

(4) Wash rapidly in dilute acetic-picric acid solution (3 drops of glacial acetic acid a few crystals of picric acid and 10 cc. distilled water).

(5) Pass the sections rapidly through water to remove the excess of picric acid and to avoid a green shade in the connective tissue.

(6) Decolorize in absolute alcohol until the sections have lost the excess of fuchsin, which will be known by the general violet color acquired by them. This step is the most delicate moment of the process and has to be performed in abundant alcohol in order to limit its duration to a few seconds. Leaving the sections too long in alcohol more or less decolorizes the nuclei.

(7) Clear in carbol-xylol, xylol and mount in balsam.

The nuclei appear intensely stained red, the cytoplasm takes a clear green or rose-yellow stain, and the connective tissue appears a very intense blue.

This method when properly carried out is without dispute one of the best differential stains, but it is difficult to execute because the alcohol acts as differentiating and dehydrating fluid at the same time. If the dehydration is prolonged too long the color of the nuclei disappears and the section appears with a uniform green-yellowish stain; if the sections are kept in alcohol too short a time the differentiation and dehydration do not take place, and the sections take a mixed purple color without any contrast.

To avoid the difficulty of keeping the stain in the nuclei Calleja[1] modified the technic using lithium carmin or borax carmin and counterstaining with picroindigocarmin. With this substitution the nuclei take a deep red color, and the path through the alcohol can be prolonged without fading the color of the nuclei. The differentiation is perfect, and the stain is permanent. Using this method Calleja has obtained beautiful slides of neoplasms.

I have been using this method for several years in sections of eyes embedded in celloidin, and I have obtained slides which demonstrate beautifully the histological and pathological structures of the eye.

Gallego introduced acetic acid as a differentiating fluid, and formaldehyd as a "viro-fixing" fluid for the sections stained with basic fuchsin, observing that after passing the sections previously stained in fuchsin through a solution of diluted formalin the stain was fixed in the nuclei and was insoluble in alcohol.

He[2] modified the trichromic methods of Cajal and van Gieson, and introduced basic fuchsin associated with eosin, to substitute for the ordinary hematoyhlin and eosin stain.

TRICHROMIC STAIN OF CAJAL MODIFIED BY GALLEGO

(1) Fix in 10 per cent formaldehyde (sections made by freezing microtome or embedded in paraffin or celloidin).

(2) Stain one minute in Ziehl's acetic-fuchsin, (Ziehl's fuchsin 10 drops, acetic acid 1 drop, distilled water 10 cc.

(3) Wash in water.

(4) Differentiation and "viro-fixation" is performed in formalin-acetic solution for five minutes (formalin 2 drops, glacial acetic acid 2 drops, distilled water 10 cc.).

(5) Wash in water.

(6) Picro-indigocarmin one minute (aqueous solution of indigocarmin 1 per cent, one part and aqueous saturated solution of picric acid, two parts).

(7) Wash in water, alcohol, carbol-xylol, et cetera.

The nuclei stain a deep red-violet; the cartilage, mucin and "Mazt-Zellen" in very intense bluish violet; the cytoplasm in clear green or yellowish green; the connective tissue an intense blue; the muscle fibers a clear green, et cetera.

This method has the advantage over the original Cajal method in that the nuclear stain is permanent after the sections are treated by formaldehyd, the differentiation is perfected by the addition of acetic acid to the fuchsin and formalin, and the alcohol completes the differentiation and dehydrates, but does not decolorize the nuclei.

VAN GIESON STAIN MODIFIED BY GALLEGO

The method of van Gieson is one of the most variable methods. Sometimes the nuclei take the stain very lightly, or appear with a red or brown color; at other times there is scarcely any noticeable differentiation between the connective tissue and the muscle fibers, and even the best sections fade in time.

The fundamental principle of the van Gieson stain and modifications of Weigert, Masson, Curtis and others, is the over-staining of the nuclei, and the staining and simultaneous differentiation with picro-fuchsin. In the method of Gallego the nuclear stain is weak, it is fixed with formalin, and the differentiation is very intense before the use of the picro-fuchsin. The technique follows:

(1) Fixation in 10 per cent formalin.

(2) Section by frozen, paraffin or celloidin methods.

(3) Acetic-fuchsin, one minute.

(4) Wash in water.

(5) "Viro-fixation" in acetic-formol, five minutes.

(6) Wash in water.

(7) Stain one minute with picro-fuchsin of van Gieson.

(8) Wash in water. Dehydration, carbol-xylol, xylol, et cetera.

The nuclei take a red-violet stain. The cartilage and mucin bluish-violet; the connective tissue intense red; the cytoplasm,

muscle fibers, red blood cells, yellow, bacteria red-violet. The stain is more constant in its results than the van Gieson, and is also more permanent.

BASIC FUCHSIN AND EOSIN STAIN OF GALLEGO

(1) Fix 10 per cent in formalin.

(2) Section by frozen paraffin or celloidin method.

(3) Stain one minute in acidified Ziehl's fuchsin, (fuchsin of Ziehl, 10 drops, glacial aceric acid 1 drop, distilled water 10 cc.).

(4) Wash in water.

(5) "Viro-fixation" in acetic-formalin.

(6) Wash in water.

(7) Counter stain with aqueous solution of one per cent eosin for one-half minute.

(8) Wash, alcohol, carbol-xylol, xylol, et cetera.

The aspect of the sections obtained by this method is similar to those obtained with hematoxylin and eosin. It has the advantage over the last method of more delicacy and beauty of the nuclear stain; the complete transparency of the sections is due to the double differentiation. The stain is almost specific for the fundamental substance of the cartilage, "Mazt-Zellen" of Ehrlich, some mucins, and bacteria are also stained.

For the study of elastic fibers I refer those interested to the work of Gallego.

AUTHOR'S MODIFICATION

I have been using the technic recommended by Gallego with a slight modification which simplifies the method. Ziehl's fuchsin in a solution of ten drops in 10 cc. of water is not sufficiently strong to stain all kinds of specimens, and many times one has to return the sections to the solution of fuchsin after their "viro-fixation" in formalin because of insufficient nuclear stain. To avoid this inconvenience the solution of fuchsin must be stronger, 15 to 20 drops for each 10 cc. of water, and added with two drops of formaldehyd, and 2 drops of acetic acid for each 10 cc. of water. With this solution of acetic-fuchsin-formalin, the stain, "viro-fixation" and differentiation takes place at the same time, and the degree of stain desired can be followed under the micro-

scope. The nuclear stain is completed in from one to five minutes, depending upon the specimens, and over-staining is impossible due to the presence of acetic acid in the solution.

This mixture of acetic-fuchsin-formalin changes color, gradually becoming darker and opaque, loses its selective properties and becomes useless in a few days. Inasmuch as the amount of fuchsin to be used daily is so small it is preferable to use fresh solution every day.

In order to continue the differentiation in this fluid, and to increase the selective properties of the stain which colors the young and old connective fibers in different grades of blue and green, a drop of acetic acid for each 10 cc. is added to the picro-indigocarmin solution. The details of the technic used by the author are as follows:

(1) Fix in 10 per cent formaldehyd.

(2) Section by frozen, paraffin or celloidin methods.

(3) Acetic-fuchsin-formalin (15 drops of fuchsin of Ziehl, 2 drops of glacial acetic acid, 2 drops of formalin, 10 cc. of water). Variable time as determined by observation.

(4) Wash in water.

(5) Indigocarmin-acetic (10 cc. of the solution of picro-indigocarmin of Cajal, 2 drops of glacial acetic acid).

(6) Wash rapidly in water.

(7) Alcohol, carbol-xylol, xylol, et cetera.

The color of the different structures is the same as in the Gallego stain. In most of the fresh specimens it will not be necessary to use the Ziehl fuchsin, since a 1 per cent solution of basic fuchsin will give the same results, but in older specimens the Ziehl fuchsin is more powerful for nuclear selection.

The first steps of my modification can be used combined with the picro-fuchsin of van Gieson or eosin as in the Gallego technic.

If some sections do not take the nuclear stain well they can be sensitized by keeping them for six to eight hours in a solution of formaldehyde at 45°C.

SUMMARY

For sections of the eyes embedded in celloidin, the method of Callaja will give excellent results.

The use of basic fuchsin associated with formalin and acetic acid and counterstained with picro-indigocarmin, picro-fuchsin or eosin, as recommended by Gallego or in my simplified technic is absolutely constant in its result, has the advantage over the original methods of Cajal and van Gieson of being more permanent, and easier to carry out.

The trichromic method of picro-indigocarmin and fuchsin is one of the best differential stains in use today, especially for the diagnosis of tumors, such as neurofibroma, myofibroma, myosarcoma, fibrosarcoma, et cetera and in tumors which have connective tissue framework associated with epithelial cells. In the squamous cell eqithelioma this method shows very beautifully the different degrees of keratinization and cellular differentiation with different tonalities of specific colors. It is also very useful for the study of blood vessels, demonstrating very well the various structures in pathologic arteries and veins, particularly in lesions of thrombo-angitis obliterans. This stain combines the advantages of hematoxylin and eosin staining and the van Gieson stain.

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EDITORIAL

The Relation of Calcium and Phosphorus Metabolism to Certain Non-Inflammatory, Non-Neoplastic Bone Diseases

The rôle of calcium in the production of bone is well recognized, but the fact that the bones act as a storehouse of calcium and other salts from which there is a continual ebb and flow received less attention up to the discovery by Collip of the active principle of the parathyroid glands (parathormone). Injection of this hormone may increase the blood calcium up to double the normal level and may increase the urinary excretion as much as ten times. Unless the ingestion of calcium is markedly increased during the administration of parathormone, the bone storehouse is depleted according to the work of Bauer, Aub and Albright, who demonstrated radiographically that the density of the bones was decreased. Hunter has shown histologically that the bone change takes place through a process of lacunar resorption by oseoclasts.

Although the most marked effects of parathormone are upon calcium metabolism, phosphorus excretion is also sharply increased and the blood phosphorus is decreased. Continued administration of the hormone has the reverse effect on the blood phosphorus.

Depletion of skeletal calcium in the form of generalized osteitis fibrosa was correlated with parathyroid disease through the accurate description of the disease by von Recklinhausen (1904), and the discovery in a typical case of a parathyroid tumor by Askanagy (1907); also through the demonstration of enlarged parathyroids in three cases of osteomalacia by Erdheim (1907), and the collection of twenty-seven cases of hypertrophy or adenoma of the parathyroids in seventeen cases of generalized osteitis fibrosa, eight cases of osteomalacia and two cases of

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rickets by Hoffheinz (1925). The question as to whether the parathyroid changes were primary or secondary arose, but Schlagenhaufer and Maresch suggested that the parathyroids be removed in generalized osteitis fibrosa. Mandl followed this suggestion ten years later (1925), finding a tumor which was excised with marked improvement of the patient's condition. In the subsequent six years more than thirty-five cases of generalized osteitis fibrosa cystica have been operated. Both the experimental evidence and clinical experience with this disease demonstrate it to be one of calcium and phosphorus metabolism in which the parathyroids have a definite etiological significance in a majority of instances at least. Hunter, and Ballin and Morse suggest the name hyperparathyroidism or parathyroidism for this group of cases.

In contrast to generalized osteitis fibrosa the localized disease is not associated with appreciable changes in calcium and phosphorus metabolism.

Rickets and osteomalacia show normal blood calcium but a low inorganic blood phosphate. Pommer and Schmorl believe that the usual calcifying mechanism for conversion of osteoid tissue to bone is impaired. Although the mechanism is not understood, it is known that vitamin D supplies the deficiency and allows the normal utilization of calcium and phosphorus.

Hunger osteomalacia, so prevalent during the war, is considered a true osteomalacia by Schmorl and Partsch and is characterized by a marked osteoporosis. In some cases hypertrophy of the parathyroids is found and vitamin D is a specific.

The osteoporosis associated with hyperthyroidism is comparable anatomically with osteomalacia, especially of the hunger type, but chemically both the blood calcium and blood phosphates are normal. The phosphatase in the blood is increased and the excretion of both calcium and phosphorus is markedly elevated.

Renal rickets and the rare rickets associated with coeliac disease, are interesting variants of this group. In renal rickets the phosphates are retained in the blood as they are in other types of renal sclerosis. The low blood calcium is considered to be secondary

to this retention of phosphates.   As opposed to true rickets there is no vitamin D deficiency. Coeliac rickets simulates true rickets in that the negative calcium balance and associated osteoporosis may be combated with vitamin D.

Further study of the calcium and phosphorus metabolism must be made in not only the non inflammatory-non neoplastic diseases mentioned but also in Paget's disease, osteogenesis imperfecta, leontiasis ossea, fragilitas ossium and perhaps others, especially with reference to the parathyroids, thyroid, posterior lobe of the pituitary, the pancreas, and the kidneys since all of these organs are known to be capable of effecting the utilization, storage, retention, or excretion of either one or both the minerals in question.

F. W. Hartman.

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Announcement has just been made by the Wistar Institute of Anatomy and Biology that beginning in 1932 a new journal

will be published to be known as the Journal of Cellular and Comparative Physiology. The journal will be issued bimonthly and each volume will contain 500 pages. The Managing Editor is Dr. E. Newton Harvey of Princeton.

Dr. Louis B. Wilson was elected President of Sigma Xi National Honorary Society at the Convention held in New Orleans.

The following University appointments have been announced:

Dr. A. F. DeGroat has been appointed professor of Pathology at the University of Arkansas School of Medicine.

Dr. Stuart Mudd has been appointed associate professor of bacteriology at the University of Pennsylvania School of Medicine.

Dr. Sydney Dalrymple has been appointed instructor in pathology at Tufts College Medical School.

Dr. Louis Tuft has been appointed associate in immunology and chemotherapy at Temple University School of Medicine.

The School of Medicine at George Washington University has recently organized a faculty in contra-distinction to the teaching staff. Among the members are Dr. Earl B. McKinley, Bacteriology; Dr. Oscar B. Hunter, Pathology; Dr. Joseph H. Rowe, Biochemistry and Dr. George B. Roth, Pharmacology.

Dr. Esmond R. Long has recently been appointed director of laboratories of the Phipps Institute.

While all legislation pertaining to medicine is of interest to clinical pathologists, certain legislation is of special interest. The following items come under this category:

(1) The Seventy-second Congress, enacted Public Law No. 744, appropriating $75,000 for the purchase of a laboratory at Hamilton, Montana for the purpose of carrying on studies and research for the prevention, eradication and cure of Rocky Mountain spotted fever. A similar sum was appropriated for construction work. (2) Private law No. 299 approved and authorized a pension of $125 a month for James C. Burke, in compensation for

disabilities contracted by him when serving as a subject for experimentation during malarial fever investigations in the Philippine Islands.

The American Medical Association through its House of Delegates is attempting to have proper Federal Legislation enacted which will cure certain evils in the administration of the Veterans Bureau, in particular in regard to the treatment of veterans suffering from nonservice connected injuries. It is further attempting to guide Congress in regard to legislation pending which will prohibit the use of live dogs in the District of Columbia for purposes of research.

In surveying the bills which have passed state legislatures during the past year none seem to be of especial interest to clinical pathologists except as follows:

(1) Bills were killed in Colorado and Illinois which proposed to require that all pupils and teachers be immune to smallpox before they attend or teach school. An attempt to extend the provisions of the vaccination law to private schools was killed in Massachusetts. Bills were killed in Missouri which proposed that vaccination should not be made a condition precedent to the admission of any person into schools and colleges. Unsuccessful attempts were made in Connecticut and New York to amend the vaccination laws.

(2) Antivivisection bills were killed in Illinois, Maryland, New York and North Dakota.

(3) A North Carolina law limits the right to perform an autopsy on a dead human body to cases (1) in which an autopsy is specifically provided for by statute; or (2) was directed by the deceased; or (3) is deemed necessary on an inquest, by the coroner or a majority of a coroner's jury; or (4) is authorized by the husband or wife, or one of the next of kin or some other person charged by law with the duty of burial.

(4) A law enacted in Michigan provides for the registration and supervision of laboratories making chemical, serologic or bacteriologic laboratory tests to aid in the diagnosis or control of communicable diseases.

Again the attention of members is called to the Annual Con-

vention to be held in New Orleans on May 6-9. The headquarters is the Jung hotel and those expecting to attend are urged to send in their reservations immediately. Rooms may be held during the meeting of the American Medical Association. Those desiring to present papers and exhibits should send their titles to the Secretary not later than April 1.

Dr. Herzberg, Chairman of the round table committee, has announced that one of the subjects for discussion at the Convention will be the relation of the patient to the individual physician, the hospital staff and the hospital, as well as methods of remuneration. In this connection the following letter sent by Dr. Herbert Fox to a pathologist is of particular interest:

Dear Dr. ——

I have thought over your matter of the directorship of a hospital laboratory, and I have come to certain conclusions that I am sending to you and naturally would be willing to have known by anyone else who is interested in the matter.

I do not believe that a pathologist to a hospital, or indeed anyone occupying an academic position, should sign a contract. A pathologist is as much a chief and consultant as any other member of the staff, consequently equivalent to any staff chief. The pathologist should be in no way subordinate to a managerial director or the board of directors other than would be the case with a surgeon, pediatrist and the like.

His duties, if they have to be outlined and interpreted, are professional matters at the discretion of the staff. The amounts charged for private work within the hospital should be settled with the advice and agreement of the staff, but need not be a fixed schedule any more than any other professional compensation is. Any member of the staff should be willing to coöperate to the extent that he would be advised and influenced by the will of the staff, in so far as hospital matters are concerned.

The amount of money paid by the hospital to the pathologist depends upon the adoption of the above policy with the expression of opinion by the staff to the managers. The amount and methods seem to be open to respectable differences of opinion. It seems to me that one of two courses may be followed.

The pathologist might receive a respectable sum for all his time and service. All private fees from within or without the hospital should go to the hospital treasury. The amount paid to the pathologist could be settled in many ways, but it should equal the average professional income of the rest of the staff; this could be estimated by secret ballot.

The other method is that the hospital pay a moderate honorarium for the pathologist to supervise all the laboratory work. He should be permitted in addition to charge for private and semi-private work within the hospital and to accept external work. The details of executing this plan should be settled by a combined judgement of the staff and managers. Whatever the arrangements they should not infringe upon the professional independence of the pathologist.

The physical property of the laboratory should be supplied and maintained by the hospital and the pathologist should be of character and training that will care for it.

Finally, the position of a pathologist must remain a professionally ethical one upon a gentleman's agreement comparable to that holding good with his associates on the staff. If he would not carry out the spirit of such an agreement he could easily evade the letter of the contract. He is a member of the professional staff and as such cannot be treated as a subordinate by managerial officers.

This letter contains my own opinion and does not involve the University of Pennsylvania or any other institution with which I am connected. ·

BOOK REVIEWS

A Text Book of Laboratory Diagnosis. By EDWIN E. OSGOOD and
HOWARD D. HASKINS. Pp. xix + 475, 1931, Philadelphia,
P. Blakiston's Son & Company, $5.00.

This text is a compilation and development of the outline
which has been used for some years in teaching laboratory diag-
nosis at the University of Oregon Medical School. It is therefore
primarily developed as a text and while it will be useful as a
laboratory manual it will be more valuable when used in a course
of instruction. In order to make it more useful in this partic-
ular, the authors have included an index of diseases and an
outline for laboratory class room work.

The subject matter is divided into two parts, the first of which
deals with the knowledge which a practitioner should have avail-
able at the bed side of the patient, while the second part deals
with laboratory methods. Since the authors have done their
major research in hematology and blood chemistry, one is not
surprised to find that these subjects are most thoroughly covered
in the book. In connection with the chapters on hematology
there are published six colored plates which are as good if not
better than anything that has yet appeared in American text
books. The authors have wisely given the colors which appear
after Wright's staining method which is probably the most
generally used in this country. The book does not deal ade-
quately with the subject of bacteriology or serology which is
evidently give as a separate course in the University of Oregon.
For this reason the text is not a complete reference book in clinical
pathology.

The sections dealing with animal parasites could stand some
revision in order to clear up certain misstatements and to correct
inaccuracies in the use of the specific names of parasites. For
instance, while some have suggested that *Trichomonas vaginalis*
and *Trichomonas hominis* are one and the same parasite, one is

certainly not justified in making the statement that chronic leucorrhea "should cause one to examine the vaginal secretion as well as the stool for the flagellate Trichomonas hominis." Further, the statement that nematodes have no intermediate hosts is obviously incorrect. The plate of animal parasites could also be revised to advantage and it would be especially desirable to state the magnification.

It would have been useful for the authors to have included the more simple Friedman test for pregnancy since it has come into so general use.

If one is justified in calling attention to these minor errors one should certainly call attention to the excellent presentation of the subject of clinical chemistry for here one can find an adequate treatment for all new phases necessary to this subject. All told the book makes an excellent addition to the clinical pathologist's armamentarium.

Approved Laboratory Technic. By JOHN A. KOLMER and FRED BOERNER. Pp. xxii + 663, 1931, New York and London, D. Appleton & Company, $7.50.

Although there are many books in the market dealing with laboratory technic, none so pretentious as this has ever appeared. The manual has been prepared under the supervision of the American Society of Clinical Pathologists and has been contributed to by various members of the Society. Each method has been submitted to various committees and groups of pathologists and therefore is in a sense a collection of "approved" methods. While one may differ with the authors as to which method should be used for a given test, nevertheless one will find in this manual adequate methods for performing almost any laboratory procedure. The manual is divided into the following sections: (1) General laboratory methods; (2) clinical pathology methods; (3) bacteriology methods; (4) serology methods; (5) clinical methods.

Even a casual glance of the chapter heads of this book will give one an idea of the enormous scope of the manual. One will not only find methods of doing almost any test necessary to perform

in a laboratory but one will also find information concerning the housing, feeding, inoculating, bleeding and autopsying of animals, methods for prevention and the emergency treating of laboratory accidents and one will also find methods dealing with milk examination, food examination, toxicological examinations and methods for the microscopic examination of tissues.

The book is profusely illustrated with excellent figures which have been beautifully reproduced. One might object to the fact that some of the color plates in hematology have been made after using Wilson's stain which is little used in this country and unfortunately the technic is omitted from the text. One may also object to the fact that the authors have not given the original source of some of the illustrations and some of the tests but in this day when literature is so profuse it is not a serious criticism.

Particularly valuable features of the book are the methods given for collecting material from patients and the exact method of procedure necessary to perform tests with good instructions for the use of the apparatus necessary and good figures.

All told the text is as near an encyclopedia of laboratory procedure as has yet appeared in this country and will not only be warmly received by clinical pathologists but will be frequently consulted by them as well as by technicians. The Society may well feel proud of having in a way had a hand in the development of this book.

ADENOMYOMA (ENDOMETRIOMA) OF THE UMBILICUS[*]

HERMAN SPITZ

Nashville, Tennessee

The endometrial nature of adenomyomas of the umbilicus was first suspected by Goddard[17] who reported two umbilical tumors of probable uterine origin in 1909. Cullen[7] confirmed Goddard's observations reporting umbilical tumors containing uterine mucosa, or remnants of Müller's ducts and later devoted[8] an entire chapter in his book to this subject in which he discussed fifteen cases collected from the literature; these included the three cases mentioned above. Of these fifteen cases, Cullen was in doubt about four, namely, Mintz's[22] second case and a case each by von Noordon,[44] Giannettasio,[16] and Wullstein;[47] but in the light of more recent investigations, these four cases are definitely included among the cases of endometrial growths of the umbilicus.

I have been able to collect fifty-four cases of adenomyoma, or as some prefer, endometrioma of the umbilicus. These are listed in chronological order in the table.

To this number, I add the following case:

Mrs. M. N. R., forty years old, a housewife, married twenty-three years, had one pregnancy twenty-two years ago; menstruation commenced at age fourteen and has always been normal. The balance of her history revealed nothing abnormal. About one year prior to being seen by her physician, this patient noticed drainage from the umbilicus commencing two days before the menstrual period and continuing throughout the menses. Enlargement of the umbilicus was noticed which gradually increased in size. The umbilicus was red and inflamed and bloody discharge was exuding when the patient was first seen. At the time of operation, several days later, the discharge had ceased, the redness and inflammation had subsided but the umbilicus was still enlarged. There

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[*] Read before the Tenth Annual Convention of the American Society of Clinical Pathologists, Philadelphia, Pennsylvania, June 7–9, 1931.

ADENOMYOMA (ENDOMETRIOMA) OF THE UMBILICUS: CHRONOLOGICAL, CLINICAL AND PATHOLOGICAL DATA

Case	Author	Country	Date	Age	Marital State	Pregnancies	Menstruation	Hernia	Pelvic Pathology	Peritoneal Attachments	Size	Sweat Glands	Smooth Muscle	Bleeding	Pain	Swelling	Duration Symptoms	Duration Tumor	Remarks
1	Villar	F	1880	40	M	0			Uterus	Pedicle attached	Egg			P				1 yr.	*
2	Wullstein	G	1893	34						Cord not attached	3 x 1.5 cm.	P						Few mos.	No hair or sebaceous glands. Tumor diffuse in rt. broad ligament. Angioma and tumor of sweat glands
3	Mintz-1	G	1890	42	M			Developed after labor		Attached to omentum	Hazelnut		P	P		P			Recurrence 4½ yrs. after removal
4	Mintz-2	G	1890	39					Myomatous uterus					P		P		3 mos.	Developed 11 months later in upper end of scar
5	Mintz-3	G	1890	45					Uterus removed 10 yrs. before	Normal	Walnut							6 mos.	
6	Green*	E	1890	50						To peritoneum		P	P	P	P	P		2½ yrs.	Skin adherent*
7	Giannettasio	I	1900	41	M	Mt						P	A	P	P	P			*
8	von Noorden	G	1901	38	M	Mt	Normal				3 cm.	P	A	P	A			2 mos.	*
9	Ehrlich	G	1909	51	M	0	Normal												Tumor of sweat glands. Bilateral mammary carcinoma
10	Goddard-1	U.S.	1900	41	S	0		None	Normal	To rectus	2 cm.	P	P	P	P	P	1 yr.	2 mos.	
11	Goddard-2	U.S.	1900	42	M	4				Abdomen opened, normal	2 cm.	P	P	P	A	A	6 yrs.		Obese
12	Herzenberg	G	1900	30	M	4		None	Fibro-adenoma of the uterus 1 mo. before	To peritoneum	2.5 cm.			P	P	P	1 mo.	1 mo.	Upper end of laparotomy scar
13	Cullen	U.S.	1912	38	M	4	Regular	None		Normal	1.5 cm.	P	P		P	P	2 yrs.	1 yr.	*
14	Waegeler	G	1913	48							Hazelnut	P	P	A	P			2 yrs.	Obese
15	Zitronblatt	G	1913	30							Filbert		P	P				Few mos.	*
16	Barker	E	1913	37				None		Abdomen opened, normal									Recurrence 4½ yrs. at left anterior superior spine of ilium*

No.	Author	Country	Year	Age	Marital	Menstruation	Umbilical	Operation	Pathology	Size					Duration		Duration	Remarks
17	Jones	U. S.	1913															*
18	Lindau-1	G	1914												11 yrs.			Above and to left of umbilicus
19	Lindau-2	G	1914	31	S					0.75 in. 17 x 20 mm.	P	P		P				Left and above
20	Mathias	G	1920	40		Normal				18 x 20 mm.	A	P		P				Goblet and bony spindle cells*
21	Guthrie*	U. S.	1920	46	M 2	Normal		Abdomen opened, normal	Normal	1.5 cm.	P	P	A	P	P		2 yrs.	*
22	Adams*	U. S.	1920	37				Abdomen opened, normal		1.3 cm.	P	P	A	P	P		1 yr.	*
23	Mahle and MacCarty	U. S.	1920	42	M 4		Umbilical hernia				P	P	P				4 yrs.	Growth not attached to umbilical hernia*
24	Keitler	G	1923	45	M 2	Normal	Nut-size, livid	Normal		Walnut	P	P	P					*
25	Lauche-1	G	1923	48	M 1	Normal		Abdomen opened, normal		Walnut		A				1½ yrs.		*
26	Lauche-2	G	1923	38														*
27	Lauche-3	G	1923	50	M 5		Small umbilical at last pregnancy	Abdomen opened, normal		3 marks	A		P			1½ yrs.		This case also reported by Schneider and by Hibbert*
28	Tobler-Case 6	G	1923	27				Tumor through wall attached to peritoneum by fossette	Fibro-myomatous uterus, cysts of ovaries and tubes, growth on cervix	2.5 cm.	P	A	P					Giant cells. (Langhans?) Serial sections show glands coming from peritoneum
29	Tobler-Case 7	G	1923	44						2 cm.	P	A	P	P	3 yrs.			
30	Andrews	E	1925	44	M 0	Painful and profuse 3 yrs.	None		Tender mass, Douglas pouch	Walnut	P	P	P	P	3 yrs.	1 yr.		Urachus normal
31	Schliffman and Seyfert	G	1925	57	M 1	Irregular 4 yr.		Normal	Papillary cystoma rt. ovary, plaque Douglas pouch	Walnut	P	P	P			10 yrs.		Emaciated. Hair follicles and few islands decidual and giant cells
32	Edwards and Spencer	U. S.	1925	45	M 3	Normal		Normal	Normal	2.5 cm.	P	P	P	P	P		7 yrs.	Obese

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Key: G—Germany, U. S.—United States, F—France, I—Italy, Den.—Denmark, Scan.—Scandinavia, B. A.—Buenos Aires, H—Holland, E—England, P—present, M—married, S—single, Mt. multipara, Mt. multipara, ✳—four miscarriages, †—abortion, A—absent, •—no clinical history.

ADENOMYOMA (ENDOMETRIOMA) OF THE UMBILICUS: CHRONOLOGICAL, CLINICAL AND PATHOLOGICAL DATA—*Concluded*

CASE	AUTHOR	COUNTRY	DATE	AGE	MARITAL STATE	PREGNANCIES	MENSTRUATION	HERNIA	PELVIC PATHOLOGY	PERITONEAL ATTACHMENTS	SIZE	SWEAT GLANDS	SMOOTH MUSCLE	BLEEDING	PAIN	SWELLING	DURATION SYMPTOMS	DURATION TUMOR	REMARKS
33	Stacy et al.	U. S.	1920	39	M	0	Increase 3 yrs.	None	Multiple fibromyomata uterus					P	P		1 yr.		Negro. Degeneration of some of the fibromyomata
34	Anglesco	I	1920	34	M	1			Cyst of ovary 2 yrs. prior				P	P	P	P		1 yr.	*
35	Weller-1	U. S.	1927	40									A	P					*
36	Weller-2	U. S.	1927	45									A	A					*
37	Oberling and Hickol[a]	F	1927	11							Cherry				P	P	Since childhood	6 mos.	
38	Steiner[1]	G	1927	46	M	Mt	Normal		Normal	Abdomen opened, to rectus and peritoneum	Hazlenut	A	P		P	P		1 yr.	*
39	Palmen-1	Scan.	1927	35	S	0	Normal							A	P	P		6 mos.	Meckel's diverticulum present, not attached to tumor
40	Palmen-2	Scan.	1927	48	M	5	Normal			To peritoneum	Egg			A	P	P		15 yrs.	Small opening into tumor from peritoneal surface. Obese
41	Palmen-3	Scan.	1927	33	M	2	Normal			To peritoneum and skin	Walnut			P	P	P		2 yrs.	
42	Lollovra and Montpellier[a]	F	1927							Pedicle at base not attached to peritoneum. Abdomen opened, normal				P	P	P			*
43	Kohler	G	1927	40	M	0	Normal							P	P	P		5 mos.	*
44	Foderl	G	1927	38	M	1	Normal			Cord not attached to peritoneum	Hazlenut	P		P	P	P		3 yrs.	*

No.	Author		Year				History	Hernia			Tumor	Uterus	Findings				Duration	Remarks
15	Baltzer	G	1927	11	M	2					Hen egg	Myomatous uterus	Abdomen opened, numerous growths through abdominal wall; not attached to peritoneum		p	p	3 yrs.	Obese. Similar growths on left ovary, appendix, peritoneum and sigmoid
16	Busse	E	1928	30				Right inguinal hernia at 15					Abdomen opened, normal		p	p	2 yrs.	
17	Roques	E	1928	19	M	8				p	17 x 15 mm.		Not connected to peritoneum. Abdomen opened, normal. Sinus in peritoneal surface		p	p	4 mos.	
18	Baltzer	G	1929	10	M	1	Recently irregular				Pear	Large uterus. Ulceration on anterior lip of cervix	Abdomen opened, numerous growths			p	8 weeks	On peritoneal surface, sigmoid, and in pelvis
19	Preye	U. A.	1929	55	M	5	Normal			p	Rosette at internal surface	Normal			p	p	1 yr or more	Both tubes contained about 10 cc. dark, old blood. Died 8 days after operation
50	Holm-1	Den.	1930	13	M	0				p	Hazelnut		To peritoneum and some into tumor			p	Some time	
51	Holm-2	Den.	1930	20		1t			?	p	Small nodule		Reentrance, adherent to peritoneum and tumor		p	p		Recurred in two months. Metastases, left supra-clavicular region, tight inguinal region. Died within a year. Post mortem shows numerous growths through pelvis, liver, etc.
52	Enzer	U. S.	1930	18			Normal				2.5 cm.		To peritoneum. Abdomen not opened	?	p	p	6 mos.	Serial sections show glands coming from peritoneum. Two glands composed of goblet cells
53	Keene and Kimbrough	U. S.	1930							p			Abdomen opened, normal		p	p		
54	Keene and Kimbrough	U. S.	1930															
55	Spitz	U. S.	1931	10	M	1	Normal			p	3 x 3 cm.	Clear	Normal		p	p	1 yr.	Similar growths in both ovaries, none in anterior abdominal wall

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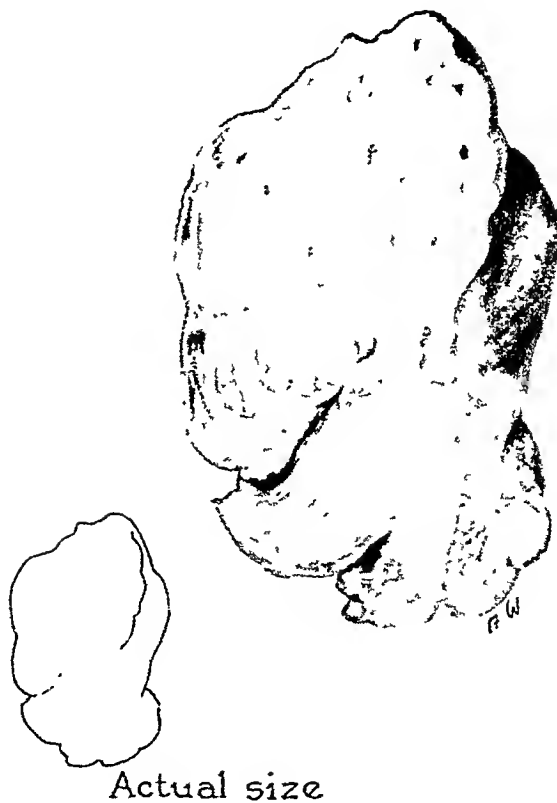FIG. 1. EXTERNAL AND UPPER SURFACE OF TUMOR

Actual size

FIG. 2. CUT SURFACE OF TUMOR SHOWING SMALL CYSTS FILLED WITH OLD
MENSTRUAL BLOOD

was no pigmentation nor could any pelvic or abdominal pathology be found. The blood and urine were normal.

The specimen (fig. 1) sent to me by Dr. Watt Yeiser of Columbia, Tennessee, was approximately 5 cm. in its greatest length, that is from its epidermal surface to its base and approximately 3 cm. in its greatest diameter. The upper surface (fig. 1) showed an elevated mass about 1.5 cm. in diameter which projected above the level of the surrounding collar of skin for 15 mm. This surface
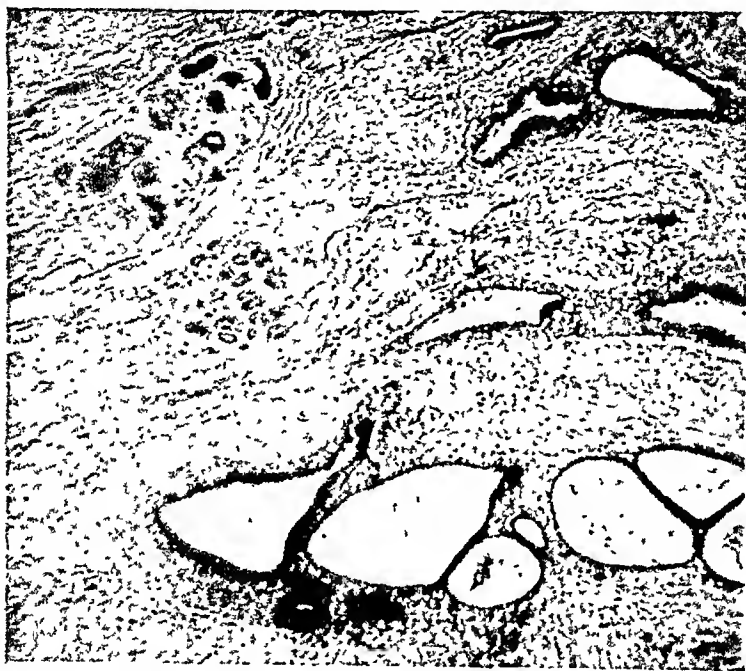
FIG. 3. PHOTOMICROGRAPH OF SECTION OF TUMOR

In the upper left hand corner note normal sweat glands. Endometrium-like glands surrounded by cytogenous tissue are seen in the upper right hand portion. The lower right hand portion shows a chain of endometrial glands undergoing cystic dilatation. Note absence of cytogenous tissue around the glands in the lower right hand corner and note contents of glands.

was covered by a dirty grayish exudate in which there were scattered a few irregularly shaped, small sized brownish masses. The sides and base of the specimen simply showed the cut surface of the surrounding connective tissue. The mass was bisected and the cut surface (fig. 2) showed from above downwards the epidermis which appeared normal. About 2 mm. beneath this, there was a rather even row of dark reddish to dark brown colored areas, varying in size from less than 1 mm. up to 3 mm. in diameter. Sections through other portions of the tissue, showed some of the above brownish areas to be prolonged down-

wards in a tubular cystic formation and from which a small amount of brownish exudate escaped. Coarse bands of connective tissue traversed the specimen in a fan-like arrangement and scattered throughout the specimen were additional brownish areas. The matrix of the specimen was smooth, creamy white and glistening.

The histological structure showed a normal epidermis which has thickened in some areas. Beneath this (figs. 3, 4 and 5), merging imperceptibly into the tumor mass were interlacing bundles and strands of connective tissue, in the meshes of which were located numerous glandlike structures which varied widely in size and shape. They were cut in cross, oblique, and longitudinal sections. Some were very small, others assumed cyst-like dimensions (fig. 4); they occurred singly, in groups of three and four or more and in chains. Many of these gland-like areas were surrounded by a heavily stained zone of round cells which contained large round nuclei. Towards the periphery of this zone of round cells, spindle-shaped cells were seen to emerge. These were arranged in small strands and groups, in cross, oblique, and longitudinal sections and blended into the surrounding connective tissue stroma. These spindle-shaped cells resemble non-striated muscle.

Immediately beneath the epidermis were several groups of small, round glands, uniform in size and shape. These were lined with cuboidal epithelium. These glands were enmeshed in a connective tissue stroma and showed no evidence of hyperplasia or hypertrophy. They were definitely normal sweat glands (fig. 3). No hair follicles were seen; an occasional mass of round cell infiltration (lymphoid cells?) was seen throughout the entire specimen.

Further consideration of the histo-pathology will be described in greater detail under the general discussion.

GENERAL DISCUSSION

Adenomyomas of the umbilicus are easily recognized new growths. They occur exclusively in women* and as a rule have

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* Koslowski[25] reported a case of true adenoma of the umbilicus in a man 55 years of age. Due to the fact that several previous investigators have included this case in their group of umbilical adenomyomas, it is advisable to point out that this tumor does not conform to any of the descriptions given of these cases The growth was located half way between the umbilicus and the symphysis. It, of course, had none of the physiological symptoms of pain, swelling and bleeding during the menstrual period (?), and the author called his growth fibro-adenoma-sub-malignun. Cullen[5] considered this growth in connection with the growths arising from the omphello-mesenteric duct, but doubts this possibility. He again considered this same case in connection with tumors of the urachus, where he finally placed it. Lauche also takes exception to including Koslowski's case with the adenomyomas of the umbilicus.

the following characteristic features: they occur during the menstrual life; are small, slowly growing, becoming swollen, tender, cyanotic and in about half of the cases discharge bloody fluid during the menstrual period. These symptoms subside with the cessation of menstruation only to reappear at the next cycle.

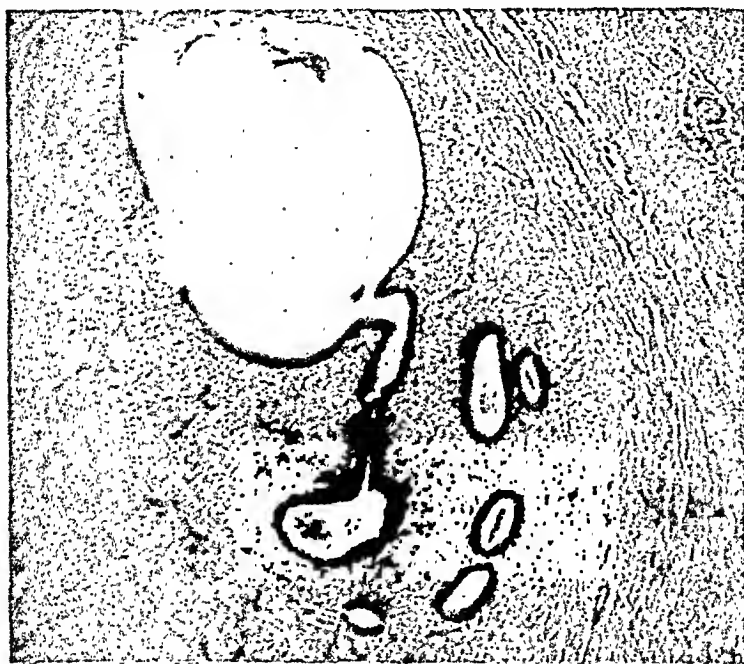Histologically, these growths are characterized by containing numerous glands embedded in a cellular (cytogenous) stroma, having the identical appearance of the uterine mucosa. Old

FIG. 4. PHOTOMICROGRAPH OF SECTION OF TUMOR

Note cytogenous tissue around small glands and absence of this tissue around the cystic gland. Also note communicating duct between cyst and smaller gland.

blood pigments, desquamated cells and debris are found in the gland spaces, especially those that are becoming cystic; extravasated blood is also seen in extra-glandular tissue spaces.

The exact origin of these growths is not known and a voluminous literature has accumulated as a result of the arguments supporting the various theories proposed to establish their origin.

These growths are similar in nature to endometrial growths found in other localities; namely, in the wall of the uterus, the ovaries, recto-vaginal septum, tubes, round ligaments, sigmoid flexure, wall of appendix and small intestine, rectus muscle, abdominal scars following Caeserian sections, certain laparotomies, and in the inguinal region and the umbilicus. Collectively, these growths have been called endometriosis. Mintz

FIG. 5. PHOTOMICROGRAPH OF SECTION OF TUMOR

Note bifurcation of gland resembling immature uterus (first described by Cullen). Lymphocytes are in the lower portion of the field and the contents of the cystic gland contains a strip of desquamated lining.

first described the growths in the umbilicus as true adenoma of the umbilicus. When involuntary muscle was identified in these growths, the term adenomyoma was applied. Blair Bell first applied the term endometrioma and this seems to be the most appropriate name. Other designations, such as fibro-adenomyosis, muellerianensis, adenomyositis, seroepitheliale adenoma, choristoblastoma seroepitheliale, and others have been used according to the personal preference of the writer. I prefer the

term endometrioma because it describes the true character of the tissue.

GEOGRAPHICAL DISTRIBUTION OF UMBILICAL ENDOMETRIOMA

Fifteen cases including my own have been reported in the United States, four cases have been reported from England, twenty-four from Germany, three each from France and Sweden, two each from Italy and Denmark and one each from Holland and Argentina.

I have not found any cases reported from the Orient, the Far East, or Africa, although numerous cases of endometriosis of the abdominal cavity and pelvic organs are reported from all quarters of the globe.

The case reported by Stacy[39] et al. was in a negro woman; all others were apparently white women.

AGE INCIDENCE

Enzer's[12] case, a girl eighteen years old is the youngest on record. The other cases reported were of the following ages: two, between twenty and thirty years of age: sixteen between the ages of thirty and forty; twenty six between the ages of forty and fifty: two patients were fifty, one each was fifty-four, fifty-five and fifty-seven. The age of the patient was not recorded in five instances.

MARITAL STATE

The clinical histories are incomplete in regard to the marital state of the majority of patients. Of the twenty-seven married women, five bore no children, seven bore one child each, four bore two children each, one gave birth to three children, three had four children each, three had five children each and one gave birth to eight children. It was stated in the case of three other patients that they were multiparas.

MENSTRUATION

Sixteen authors stated that menstruation had always been normal. Irregularity attended with pain and profuse bleeding

for three years was reported by Andrews;[1] Schiffman and Seyfert[38] reported four years with pain and profuse bleeding; Stacy et al., 3 years; Baltzer's[4] second case, recent irregularity. Holm's[21] second case, had one abortion; Roques,[37] had four miscarriages. The other case records contain no data in regard to the menstrual condition.

The majority of writers make no observation about their patient's constitutional resistance. Goddard's second case, Edwards and Spencer's,[10] Baltzer's[3] first case, Palmen's[35] second case, and Waegeler's[45] case, were obese. Schiffman and Seyfert's case was emaciated.

<div align="center">HERNIAS</div>

Mintz's first case developed an umbilical hernia shortly after pregnancy and the umbilical growth commenced ten years later. Keitler[24] reported a nut-sized livid hernia. Roques reported a right inguinal hernia, at the age of 15, thirty-four years previous to the development of the tumor. Lauche's[27] third case, which is also reported by Ribbert and Schneider in 1916, had a small umbilical hernia which developed at the last pregnancy. Mahle and MacCarty[30] report an umbilical hernia and state that the tumor was not attached to it. The absence of a hernia was specifically noted by Goddard in his second case, by Barker,[5] Stacy et al., Schiffman and Seyfert and Waegeler. The others do not mention hernia.

<div align="center">ABDOMINAL AND PELVIC GROWTHS</div>

Baltzer found generalized endometriosis in both of his cases. The first case had growths on the peritoneum, appendix, sigmoid and left ovary; a myomatous uterus was also present. His second case had similar growths on the peritoneum, sigmoid and in the pelvis. As a result of these observations, he strongly advised that the abdominal cavity be thoroughly explored in every case.

The abdomen was opened in Mintz's second case in which a myomatous uterus was found and an umbilical growth developed eleven months later; it was firmly attached to the omentum.

Ehrlich's[11] case had a uterus removed ten years before the growth developed. Wullstein's case had a pelvic tumor the size of a fist connected to the uterus and diffuse growth through the right broad ligament. The umbilical growth had a pedicle extending into the abdominal cavity. This was not connected to the pelvic growth. Herzenberg's[20] case had a fibro-adenoma of the uterus. The umbilical growth developed one month later in the upper end of the laparotomy scar. Andrew's case had a fixed tender mass in Douglas' pouch. The umbilical growth was not connected to this mass but sections from both growths showed the same structure. Schiffman and Seyfert's case had a cysto-papilloma of the right ovary and a white, flat, elevated plaque in Douglas' pouch. There was no connection to the umbilical growth. Anglesio's[2] case had a cystic ovary removed two years before the appearance of the umbilical growth. The case reported by Stacy et al. had large multiple fibromyomas of the uterus, some of which were degenerating and adherent in the pelvis. The umbilical growth was not attached. Frero's[15] case showed an ulceration of the anterior lip of the cervix; the uterus was large and hard and both tubes contained old, dark blood; they were adherent to the posterior face of the isthmus and Douglas' pouch; the umbilical growth was not attached. Holm's second case, recurred within two months and developed masses in the right inguinal region and the left supraclavicular fossa. Sections were the same as in the original growth; the patient died within a year and similar growths were found in the liver, posterior mesenteric glands, along the left ureter (which was greatly dilated above the point of pressure by the growth), a mass the size of a fist in the supraclavicular fossa, in the groin, and the umbilical growth had grown through the entire thickness of the abdominal wall and was adherent to the omentum and peritoneum. Tobler's[42] seventh case had numerous small cysts of both tubes and ovaries and multiple fibromyomas of the uterus. Keene and Kimbrough's[23] first case had similar growths on both ovaries.

Fraser[14] reported an umbilical endometrioma with twenty-three distinct similar growths in various places in the pelvis and peritoneal cavity in a monkey, *Macacus rhesus*. This is very

similar to the two cases in humans reported by Baltzer. It may also be noted here, that generalized endometriosis in the peritoneal cavity without an umbilical growth has been reported by several writers.

The abdomen was opened in cases reported by Goddard (second case), Barker,[5] Mahle and MacCarty, Keitler, Kohler,[25] Roques, Busser et al.,[6] Lauche (second case), and Keene and Kimbrough with normal findings. Palmen's first case had a small Meckel's diverticulum which was not attached to the umbilical tumor.

PERITONEAL ATTACHMENT

Attachments to the peritoneum by means of cords or stalks were reported by Villar,[43] Foderl,[13] Palmen in all three of his cases, Waegeler, and Holm's first case. It was connected to the omentum in Mintz's second case, and in Holm's second case. Stalks or cords were present which penetrated the abdominal wall to the peritoneum but were not attached in Wullstein's, Foderl's and Kohler's cases. In Palmen's first case, in which the small Meckel's diverticulum was present, the umbilical growth was attached to the peritoneum and rectus sheath; in his third case the growth was adherent to the skin and peritoneum. The growth occupied the entire thickness of the abdominal wall in Baltzer's first case, Holm's second case, and Tobler's seventh case.

DURATION OF SYMPTOMS AND TUMORS

Symptoms such as uneasy feeling, itching, pain, tenderness, swelling, redness and cyanosis were present in a number of cases prior to the development of the tumor. The shortest length of time reported was two months while the longest was nine years. Palmen, in his second case, reported symptoms present since childhood, the patient being forty-eight years old at the time of operation.

The tumor was noticed as early as one month prior to operation in one case. In thirteen cases prior to one year; in fourteen cases, one to two years prior; in four cases, two to five years prior; in

one case, seven years prior; in one, ten years and in one, fifteen years prior.

There was a recurrence after four and a half years in Mintz's first case in the umbilical scar.   In Barker's case, four and a half years after removal of the umbilical growth, a nodule appeared at the anterior superior spine of the ileum.   In Holm's second case, within two months the growth recurred locally and metastases were present in the right inguinal region and left supra-clavicular fossa.

Pain, swelling and discharge of bloody fluid were present in the majority of cases.   Twenty-five had discharge of bloody fluid from the growth.   Six had discharge of sanguinous fluid without attendant pain and swelling.   In sixteen cases pain and swelling was present without discharge.   The appearance of these symptoms was not regular.   In some the discharge would appear at regular monthly intervals while in others it might be profuse one month and absent for several months and then reappear.   Some would have pain and swelling without bleeding and the following month have the discharge without pain and swelling.   The majority would notice pain and swelling with the development of redness or cyanosis around the umbilical scar for several days prior to the onset of menstruation followed by discharge of varying amounts from the growth, from a few drops to several teaspoonsful, of light stained to almost black blood.   The discharge would subside with the menstrual flow (some discharging a varying number of times), this would be followed by the disappearance of the swelling, redness or other discoloration, and pain.   In some, a greenish discoloration of the umbilical region would remain for several days.   After a period of quiescence, the cycle would commence again.

GROSS APPEARANCE OF THE GROWTH

The majority reported a single nodule or tumor lying deep in the umbilical scar, filling it and in some cases projecting to a variable distance (1 or 2 cm.) above the level of the surrounding tissues.   Several reported two nodules and a papillomatous appearance.   Pinpoint openings, through which the discharge is

seen to escape, are reported by some. Several reported a thin, tissue paper like covering over a cyst like cavity, through which the blood contents of the cavity can be seen. The shape of the tumor is not definite. None are encapsulated; the majority merge gradually with the surrounding connective tissue, although in some the tumorous mass can be more readily identified. Some are covered with a dry, bloody secretion; my case was covered with a purulent like exudate. As noted above on peritoneal attachments, some of these growths occupy the entire thickness of the abdominal wall. As a rule, they are movable; it was firmly adherent to the skin in only two instances (Palmen's third case, and Mintz's third case). Even when attached to the rectus, they are still movable.

Some of these growths were excentric and appeared to one side or the other of the umbilical scar. In several cases they were located in the upper end of the laparotomy or hernial scar. Several specimens had definite sinuses at the upper or in the lower poles and as noted above, cord-like attachments to the peritoneal surface were present in a few of the cases. The cut surface of the growth was described almost uniformly as consisting of a pearly or creamy grayish appearance with coarse trabeculae or fasciculae of radiating, for example, fan shaped bands of connective tissue coarsing through the specimen. Brownish or reddish brown areas were seen scattered throughout the specimen, few to numerous, small pinpoint to several millimeters in size. In several cases larger cyst like cavities were noted. Sinuses running for considerable distances through the specimen were noted in a few cases.

HISTOLOGICAL APPEARANCE

The histological appearance is characteristic and with few exceptions, which will be noted, are identical in all specimens. Photomicrographs accompany many of the articles and an endometrial like appearance is clearly shown. The skin is hypertrophied, the papillae deepened, the basement membrane smooth and intact. Brownish pigment and deposits of red blood are frequently reported in the deeper layers of the epidermis. Fibrous connective tissue, arranged in course bands and in fine fascicula-

tion, traverse the sections. At numerous places gland like structures appear. These vary in size from a pinpoint to cystic dimensions. Smaller glands are frequently arranged in groups or chains and cysts are frequently seen lying side by side. The glands are seen in cross, oblique and longitudinal sections. These glands are surrounded by a compact cellular stroma composed of round and spindle-shaped cells with large, round or oval, deeply staining nuclei. This stroma, in various places, blends gradually with the surrounding fibrous connective tissue in some, while in others, the line of demarkation between the two types of tissue is clear cut.

This typical stroma was first called "cytogenous" by Ribbert and practically all of the German writers have adopted this term to describe the stroma surrounding the glands. This stroma is also interesting in that it bears a definite relation to the glands and will be referred to later.

The lining of the glands varies with the size and cystic proportions. The small glands are lined with high cylindrical epithelium with the nucleus situated from the middle to the base of the cell and many of these cells have cilia. As the glands get larger, that is become cystic, the lining cells first become cuboidal and in the largest cysts, the lining cells are flat. Many of these cystic areas contain (fig. 5) desquamated epithelial cells, some of which are of recent origin, while others are old. They also contain old and fresh blood, blood crystals and amorphous debris. Other cysts are empty. Most of the writers interpret the change and the shape of the lining cells as being due to pressure from the cyst contents. Occasionally, a group or chain of glands are seen which gradually increase in size and a communicating duct can be seen between some of them (fig. 4). The "cytogenous" stroma, is especially well developed and present in large masses entirely surrounding the smaller glands, which are lined with cylindric epithelium, but as the epithelium assumes the flatter shape, the "cytogenous" stroma gradually becomes thinner and thinner, until it is entirely absent surrounding the portion of the cystic spaces lined with flat epithelium. Ribbert refers to this condition as the "floor of the cyst resting upon a foundation of

heavy cytogenous stroma, while the roof has only fibrous connective tissue to cover it." Capillaries and larger sized blood vessels, together with lymph spaces are present. In many, small to larger masses of lymphocytes are present. Evidence of old menstrual blood and also of recent hemorrhage is found in many portions of the tissue.

Two other elements are frequently present: these are sweat glands (fig. 3) and involuntary or smooth muscle from which the tumor derives a portion of its name. Cullen says, "the normal umbilical scar is covered with a very thin squamous epithelium and is devoid of hair follicles, sweat glands and sebaceous glands."

Of the twenty-five cases having a discharge smooth muscle and sweat glands were present in four, smooth muscle alone was present in eight, sweat glands alone were present in three, neither smooth muscle nor sweat glands present in ten. Sweat glands and smooth muscle without discharge were present in five. It should be remembered that microscopic evidence of either old or fresh hemorrhage, usually both, was present in almost every instance.

In Wullstein's case an angioma was present, with a marked increase of sweat glands; hair follicles and sebaceous glands were absent. Mintz's second case contained collagen fibers. Ehrlich's case had a tumor of the sweat glands; he specifically states goblet cells are not present. Mathias'[31] case contained a few goblet cells and some bony spindle cells. Schiffman and Seyfert reported hair follicles, a few islands of decidual cells and a few giant cells. Enzer[12] reported two glands consisting entirely of goblet cells. Tobler (case 6) reported granulation tissue and foreign body giant cells, which reminded him of Langhan's cells. Holm (second case) reported occasional mitotic figures in the sections from his post mortem material; nowhere could he discover a breaking through of the basement membrane; sections from all the areas (umbilical, inguinal region, supraclavicular region, omentum, liver, et cetera) showed the typical endometrial structure.

THEORIES REGARDING ORIGIN

The earlier writers considered the remains of the omphellomesenteric duct as being the source. Several speak of the rem-

nants of the vitalline duct and of Müller's duct, this later source being especially favored by Cullen.[7]   Sampson[39] in studying the entire question of endometriosis, reported his findings as a result of numerous observations and advanced the Theory of Implantation.   He divides the tissue into four, or possibly, five groups.

(1) Direct or primary. These result from direct invasion from the uterine wall by the uterine mucosa. These are the uterine adenomyomas.

(2) Peritoneal implantation from retrograde menstruation. These spread like cancer and then invade the underlying structures. (It seems that Baltzer's two cases might be included in this group.)

(3) Transplantation. Those that appear in abdominal scars after Caeserian section and other operations upon the uterus and tubes and after certain herniotomies. (Such growths have appeared in abdominal scars after appendectomies. Certainly, no source from the uterus or adnexa can be concerned here.)

(4) Metastatic. These are extraperitoneal and spread like cancer, possibly through the lymph and venous channels.

(5) Developmentally displaced. Though he admits the possibility, he has never seen it.

At the meeting of the American Gynecological Society, in 1925 Sampson stated:

Furthermore, this misplaced endometrial tissue is governed by the same natural laws, in its reaction to menstruation, pregnancy and the menopause, as the mucosa lining the uterine cavity. On the basis of its histological structure and physiological function, we must conclude that this tissue is as truely 'Müllerian' as that arising in the uterine wall from its direct invasion by the uterine mucosa.

Much opposition has arisen to many of Sampson's contentions. Lauche advanced the theory of peritoneal or serosal source for these various endometrial growths and he is ably supported by Nicholson,[34] and others. At the present time the question remains unsettled.

In the case of the umbilical endometriomas, I feel that Sampson's theory is untenable. I cannot conceive of these growths occurring without having some demonstrable connecting link with the parent source. Granting that laparotomies were performed in Mintz's second case, three years prior, in Ehrlich's case ten years prior, in Herzenberg's case one month prior, and that herniotomy was done ten years prior in Mintz's first case, thirty-

four years prior in Roques's case, and ovariotomy was performed two years prior in Anglesio's case, I can see no possible connection nor do these authors see any connection between these operations and the subsequent development of the umbilical growth.

As regards the transplantation theory, it of course, can be considered only in connection with operations upon the uterus and tubes. Unless the uterus is opened, as in the case of Caeserian section, I cannot conceive of the endometrial cells from the uterine mucosa reaching the umbilicus. I certainly agree with Lauche, Nicholson and others who take strong exception to this possibility. Considering the peritoneal fossettes as described by Cullen[8] the presence of which is reported by Frero, Roques, Tobler, and Enzer; the cords which were attached to the peritoneum as reported by Villar, Wullstein, Palmen's (third case), Kohler, Foderl and the presence of sinuses by Roques and Holm, one must consider the greater likelihood of these growths arising from peritoneal cells.

By means of serial sections Tobler and Enzer described the source of the endometrial like glands in their umbilical growths as coming directly from the peritoneum. Indeed, it has been shown by Nicholson, Lauche, and others, that the peritoneum is directly connected to these growths in every situation in which they have been found, possibly excepting the uterine adenomyoma, and here even, the possibility is by no means remote.

Lauche, Nicholson, Baltzer,[3] Foderl, Schiffman and Seyfert and others contend that the presence of ovarian hormones is necessary for these growths to develop. They occur only in sexually mature women and the physiological symptoms of swelling, pain and bleeding are present only during the menstrual cycle. The spontaneous disappearance of endometrial growths from the pelvis and bladder after removal of the ovaries has been reported by Graves[18] and Keene.[22]

Neumann[33] transplanted endometrium of the rabbit into the peritoneum of the same animal and of other animals from the same litter. These transplants formed cysts when ovarian hormones were present. When castration had been done a few weeks before operation, no cysts were formed and the transplants were resorbed. Transplantation into males was unsuccessful.

SUMMARY

(1) An additional case of adenomyoma of the umbilicus is reported.

(2) The geographical distribution of all the cases heretofore reported is given and the cases are arranged in chronological order.

(3) Age, menstrual history, marital state, number of pregnancies, the association of other tumors and previous operations of each case, are considered.

(4) Clinical symptoms, such as pain, swelling, discoloration, discharge and size of growth are tabulated; as are also the histological findings in regard to the endometrial like glands, smooth muscle, sweat glands and other constituents of the growth.

(5) Some of the various theories of origin are considered.

(6) Those cases showing definite cord like attachments or other direct evidence of attachment to the peritoneum are discussed in connection with the serosal theory of origin.

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THE SPECIFICITY OF BACTERIAL ALLERGY*

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The resemblance between the two phenomena appeared at first to justify the idea that allergy in human beings is identical with experimental anaphylaxis.   But as studies progressed certain discrepancies between the two were observed and the doctrine that clinical allergy was basically different from anaphylaxis gained rather wide acceptance. . The pendulum is however swinging back and with increasing evidence there appears more and more justification for the assumption that the two conditions are the same.

Curiously enough, somewhat the reverse of this sequence is taking place with regard to bacterial allergy.   Much of the groundwork on anaphylaxis had been done with bacterial antigens[6, 8, 13, 15] and the conception of sensitization to foods, pollens and the like grew out of the analogy to bacterial anaphylaxis. Recent observations on bacterial allergy indicate however a difference in the type of reaction which cannot yet be entirely fitted in to the familiar picture of anaphylaxis as we have known it in the laboratory.

Typical anaphylactic phenomena including acute anaphylactic shock, passive sensitization, antibodies and precipitins in the blood, contraction of the sensitizied uterus and antianaphylaxis, may be produced with bacterial antigens.   But two observations which have of late received especial study remain to be entirely satisfactorily fitted into the picture.   These are the tuberculin type of reaction and the discovery of the soluble specific carbohydrate substance described by Heidelberger and Avery.[5]

That the tuberculin reaction is not dependent solely on the

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presence of antibodies in the sensitized animal is indicated by the fact that it is only present in animals which in addition to being sensitized also carry or have carried active tuberculous infection. It has therefore been postulated that the delayed, twenty-four hour reaction to bacterial substance or extractives such as tuberculin depends upon the existence of a socalled "third substance" of as yet unknown nature which is manufactured by the tissues at the site of the local infection.[16] The delayed positive reaction to antigenic bacterial substance therefore indicates not only an actual sensitization but also an infection, with some as yet underdetermined specific reaction between host and parasite.

The "soluble specific substance" appears to be a complex carbohydrate, different for different bacteria, and responsible for the specificity of bacterial antigen.   The specificity appears to be so closely bound up with the carbohydrate moiety that the latter alone, freed from protein will produce a typical immediate skin reaction, with wheal and erythema, in sensitized animals.   This substance apparently determines the specificity of the immune reaction.   It combines with antibodies, yet it appears to have no antigenic power.   It is unable to initiate the production of antibodies.   It therefore appears to be a true haptene.   It is a factor in sensitization and immunity and is apparently responsible for specificity, but, alone, it will not produce sensitization.   To do this it must be combined with the protein fraction of the bacterial cell.

We must conclude that in bacterial sensitization we are studying a distinctly more complex problem than that of allergy to the usual exogenous allergens; that we are dealing with substances and reactions of whose nature we are still rather ignorant.   With these facts in mind, we are in a better position to analyze the clinical observations that have been made for and against bacterial specificity.

THE CLINICAL ALLERGIES

Among the leading protagonists of specific bacterial allergy we should mention Thomas, Famulener and Touart[12] and Brown.[1] The former believe in the specificity of the skin reaction to bac-

teria and, using bacteria to which positive skin reactions have been obtained they report complete relief in 51 per cent of their bacterial allergics and 39 per cent of partial relief. Brown reports 65 per cent complete or practically complete relief in his bacterial asthmatics. Rackemann[8] reports nearly as good results, with 18 per cent cured and 52 per cent improved, but he is by no means as certain that the reaction and the improvement are based on specific bacterial sensitization.

In my own investigation of bacterial allergens I have in general followed Famulener's technic of cultivation in dextrose broth and on blood agar and Huntoon's medium, methods which tend somewhat to promote differential growth of the streptococci, pneumococci and gram-negative organisms, with subsequent plating and pure culture isolation. At the same time I make a group isolation of those organisms which will survive twenty-four hours in the patient's clotted blood, following the Solis-Cohen selective pathogen technic. The latter in our experience appears to favor the growth of the streptococci although not exclusively. The patient is then tested intracutaneously with the differential pure culture vaccines and the pathogen mixture. Our experience coincides with that of Thomas and his collaborators and of Rackemann, and of Walker,[14] that the most frequent positive reaction is the delayed reaction of inflammatory type, appearing within twenty-four hours, but that occasionally one observes a characteristic immediate reaction with wheal and erythema.

Our results have however not been as satisfactory on the whole as some of those reported, in that in those cases showing positive prompt or delayed reactions attempts at desensitization often fail to give relief. In these same cases subsequent treatment with a pooled vaccine, a hodgepodge of those vaccines which have helped in other cases, has sometimes given relief. Furthermore as good results are sometimes obtained by such nonspecific methods as the subcutaneous injection of peptone solution.

SPECIFICITY

Such observations naturally raise the question as to the specificity of the reaction even when improvement results. Of course one must realize that in these studies we are dealing with a par-

ticularly difficult type of allergic individual in that bacterial studies are usually conducted on the residue cases, the groups which have failed to show evidence of sensitization to the more common food and inhalant allergens.   They are the group that Rackemann has very aptly called intrinsic allergics.   They are the group that are classed together simply because we have been unable to find an extraneous cause.   Some of them may actually be due to extrinsic causes which we have failed to find and the mere absence of an extrinsic cause does not prove that they are all bacterial.

Furthermore this group of intrinsic cases, particularly those of asthma and vasomotor rhinitis, usually have quite a variety of associated nonspecific changes; bronchiectasis, emphysema; polyps and the like which complicate the picture.   Even the associated bronchitis and sinus infection may act nonspecifically whether or not there be a specific sensitization to the causative bacteria.   In my experience it is not the percentage of satisfactory results with vaccines so much as the occasional startlingly good results in the individual case that leads one to feel that there is a basis for bacterial allergy.

Best results appear to be obtained when the injection of a vaccine produces a local subcutaneous reaction.   This has been cited as evidence that the response may be nonspecific.   Like Rackemann we have observed that certain of the gram-negative organisms give positive reactions to endermal tests so frequently that one begins to question their specificity.   But the following case is difficult to explain on either of these tenets.

A young lady was found to give a very strongly positive delayed reaction to an autogenous pure culture of *Pseudomonas aeruginosa* (*Bacillus pyocyaneus*). The following day the inflammatory reaction covered an area roughly three by seven centimeters.   This reaction was accompanied by a severe exacerbation of her asthma.   The pure culture vaccine was diluted 1000 times and desensitization with this dilution, which failed to give positive intradermal or subcutaneous reactions, was carried on up through the higher concentrations until she finally received the undiluted vaccine without local reaction and without asthma.   In this case the same procedure for desensitization was followed as is employed in pollen desensitization.   No local reaction was produced, and the patient was relieved of her asthma.

The exacerbation of attacks of asthma following injections of autogenous vaccine might be interpreted as evidence for specificity were it not that in a given case different vaccines, even though giving negligible endermic reactions will do the same. The exacerbation appears to be a factor of the patient rather than of the vaccine.

With occasional exceptions such as that just cited, my own experience is in agreement with that of Rackemann and of Thomas, that better results are to be anticipated if treatment results in a low grade local subcutaneous reaction. In those cases in which this is requisite the question is again raised as to the specificity of the response.

In this connection the experiments of Mackenzie and Fruhbauer[7] are of interest. They immunized rabbits against egg white until the serum precipitin titer was high. After several months the titer of this egg white precipitin had fallen almost to zero. At this time the injection of typhoid vaccine caused a reappearance of the egg white precipitin which again increased to a high titer. We must infer from these experiments that the injection of a new, foreign, nonspecific antigen will increase the production of other different but specific antibodies. This observation gives us an intelligible clue in our attempt to explain the improvement following nonspecific protein therapy.

Even in those cases such as the one cited above, which improve although there is no local subcutaneous reaction, we cannot prove from present knowledge that the results are not due to a similar type of response.

ARTHRITIS

The question of bacterial allergy is still further complicated by the altogether different type of response to vaccine treatment in chronic arthritis. Swift and his collaborators[11] have produced strong evidence that the joint manifestations of rheumatic fever are essentially allergic and the work of other investigators particularly Crowe,[2] Small[10] and Freiberg and Dorst[3] point strongly to an allergic joint factor in chronic arthritis. My own study of chronic arthritis has followed the general line developed by Crowe.

Our desensitizing vaccine has usually been derived from the colon and in different cases has been either Crowe's stock organisms (*Streptococcus arthriticus* and *Micrococcus deformans*) or an autogenous enteropathogen made up following the Solis-Cohen selective pathogen technic. In an analysis of 100 cases carefully followed over a three year period with vaccine treatment alone we found that 10 per cent had experienced complete remission of symptoms, 37 per cent had obtained a measure of relief which was considered satisfactory, 25 per cent showed some improvement, not enough to be considered satisfactory and the balance remained unimproved. Forty-seven per cent therefore received a satisfactory measure of relief during the period of observation.

The observations of this series that are of present interest and are in contrast to those which we have discussed with the more obvious clinical allergies, are as follows:

In those cases where we have made skin tests with the vaccines, we usually did not observe significant positive reactions either immediate or delayed and no reactions that were of definite prognostic value.

Successful therapeutic results depend upon the complete avoidance of reactions, either local, focal or general. The occurrence of a focal reaction after a vaccine treatment is interpreted as indicating that we are dealing with an organism of etiologic significance but for amelioration of symptoms the dosage must be cut down to where the patient feels better rather than worse after treatment.

In other words, the delayed tuberculin type of reaction appears to be less important, and relief of symptoms appears to depend upon a method of treatment more closely akin to the method of desensitization in experimental anaphylaxis. The evidence suggests that in arthritis associated with bacterial allergy we are dealing with a condition more nearly identical with experimental anaphylaxis, one in which the "third substance" responsible for the tuberculin type of reaction plays much less of a part. If this be true one would expect to find antibodies in the circulating blood. I am not acquainted with any work that has been done on the identication of specific precipitins in the blood of arthritis

but if the work of Hadjopoulos[4] and Burbank on complement-fixation tests for streptococci in arthritics is confirmed, this will give some substantiating evidence.*

In mentioning the need for extremely small dosage of pathogen in the treatment of arthritis, I would emphasize that in a measure, poor results from vaccine treatment of various diseases has been due to failure to recognize the difference between immunization and desensitization. When we are immunizing against a bacterium which may at some time in the future enter the body, experience has shown that large and increasing doses produce best results. Typhoid vaccine serves as an example. When on the contrary the organism has already invaded the system and the host is already sensitized, minute and carefully graded dosage is requisite.

CONCLUSIONS

It becomes apparent from the preceding discussion that our knowledge of clinical bacterial allergy and vaccine treatment is still quite obscure on many points. The factors which enter into the reaction are certainly more complex than those of pollen or other inhalant allergy apparently due primarily to the fact that while with the latter we are dealing with an extrinsic allergen which is absorbed into the system only on occasion, with the former we are dealing with an intrinsic allergen which is constantly present. It appears to be its presence and activity in the body that is responsible for the tuberculin type of reaction, a phenomenon whose explanation will help much in our understanding of bacterial allergy. We are dealing not alone with an antigen-antibody reaction but also with a concomitant specific infection. Successful treatment must therefore do more than merely desensitize against the specific allergen but must also in some way eradicate the focus of living organisms which are responsible for the continuation of the reaction between themselves and the tissues of the host.

The evidence to date indicates that bacterial desensitization is a

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* The recent work of Nicholls and Stainsby on agglutinins in chronic arthritis gives most important additional evidence in this regard.

rational procedure and therapeutic results in appropriate cases while not spectacular, justify the procedure. We cannot however hope for any outstanding advances until after the problems just discussed have received further elucidation.

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ACTIVE IMMUNIZATION METHODS AGAINST ACUTE DIFFUSE PERITONITIS*

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Active peritoneal immunization against a possible inflammatory process of the peritoneum is definitely established both by experimental procedure and clinical application.[12, 3, 15, 4, 7, 9] There is a concurrence of experimental data that peritoneal protection can be achieved. As is true with active immunization in general, there is lacking an efficient antigen and further knowledge of some of the basic principles. The mechanism of peritoneal immunity is apparently still controversial. There is disagreement as to whether the immunity is local as understood by Besredka (Herrmann[4]) or is merely a local manifestation of a general immunity (Steinberg and Snyder[16]). There is also doubt as to the specificity of such an immunization. However, there are sufficient data to indicate that the process is not specific. Goldblatt and I[15] immunized animals with colon bacilli and induced fecal peritonitis (the feces contained several species of organisms) with a consequent survival of the animals. Morton[5] used nonspecific substances and secured a peritoneal immunity against a hemolytic streptococcus. It is questionable, however, if Morton obtained invariably a peritonitis in his experimental animals. As I[11, 13, 14] repeatedly pointed out, introduction of bacterial cultures intraperitoneally may not produce peritonitis. The bacteria pass rapidly from the peritoneal cavity into the circulation and the animal may die or survive depending upon the virulence of the organism, but there may be no evidence of peritoneal inflammation. Employment of such a method to induce experimental peritonitis may lead investigators to erroneous conclusions.

If the peritoneal immunization is of a nonspecific nature, the employment of a single good antigen is preferable, since it lends itself to a more ready standardization. Herrmann[4] failed to secure as good results with *Escherichia coli* (*B. coli*) alone as with a mixture of *Esch. coli* and streptococcus vaccine. My[9] results were contrary to his. Probably, the colon bacillus that I use (our type number 300) happens to be of a better antigenic value.

The rapidity with which the peritoneum can be protected is of significance to the clinician. The quicker the immunity is produced, the more applicable can the procedure be made for the patient. Goldblatt and I,[3, 15] found in our early experiments that an adequate immunity could be established ten to fifteen days after the last immunizing dose. Later, the time interval was shortened and it was disclosed that survival of the experimental animals with an otherwise lethal peritonitis could be accomplished on the same or the following day after the last immunizing dose.[9] Four injections of heat-killed bacteria intraperitoneally on successive days resulted in a large percentage of survivals. Even a single injection induced a protection in some animals. A study of the peritoneal bacterial and cellular reactions led me to conclude that the protection obtained was due to a coincident presence of polymorphonuclear phagocytes in the peritoneal cavity.[10] The phagocytes persisted in the peritoneal cavity for twenty-six days. Previous experiments[8] showed that two factors were responsible for the survival of the animal with peritonitis: (1) rapid phagocytosis and (2) a sufficiently large number of polymorphonuclears to cope with the invading bacteria. The availability of the phagocytes in intraperitoneal immunization, in view of the above conclusions, is probably an important factor in the survival of the animal when the peritonitis is induced several days after the introduction of the vaccine.

It may be stated that the degree of active immunity achieved, with other factors being constant, varies with the antigenic potency of the vaccine. It is fairly generally accepted that living bacteria constitute the best antigen while heat-killed organisms make the poorest. It becomes evident that an incorrect picture may be obtained in the experimental determination of the other

factors (route of vaccine administration, type of organism used, time interval between vaccination and onset of disease) involved in active immunization if the vaccine is of poor antigenic value. Appreciation of the relative inefficiency of heat-killed bacterial vaccine prompted investigators to employ living organisms. Castellani,[2] Pescarolo and Quadrone[6] used living or attenuated typhoid and paratyphoid bacilli for active immunization against the respective diseases. Besredka[1] introduced sensitized vaccine which is a bacterial culture treated first with an homologous immune serum and later killed by chemicals.

TABLE 1

FACTORS CONCERNED IN THE ESTABLISHMENT OF PERITONEAL PROTECTION
AGAINST EXPERIMENTAL ACUTE DIFFUSE PERITONITIS

1. THE INDIVIDUAL ANIMAL, ITS CAPACITY TO PRODUCE ANTIBODIES
    a. Quantitative factor (amount of antibody)
    b. Time factor (the rapidity of production of antibodies)
2. THE ANTIGEN
    a. Quality of antigen (measured in degree of antibody response and survival of animal following infection)
3. THE TIME INTERVAL BETWEEN INTRODUCTION OF ANTIGEN AND ONSET OF INFECTION
4. THE SEVERITY OF INFECTION
    a. Qualitative factor (virulence of organism and presence of toxin)
    b. Quantitative factor (the number of organisms introduced at a given time)

The experimental evaluation of a method of peritoneal protection was found to be dependent upon several factors. The capacity and the rapidity of the individual animal to produce antibodies varied with some unknown inherent cause or with some previous antibody stimulating disease (see table 1). It was possible to gauge approximately this variable individual antibody response by a single intraperitoneal injection of a standardized colon bacillus vaccine and hourly peritoneal leukocyte and bacterial counts for twenty-four hours. The severity of the experimentally produced peritonitis could be made constant. Three billion bacteria of a twenty-four-hour plain agar culture of colon bacillus (culture No. 300) suspended in 2.5 per cent gum

tragacanth in saline constituted the fixed peritonitis producing material. In one set of animals, the peritonitis producing material consisted of 5 grams of dog feces from the small and large bowel, suspended in 40 cc. of saline. Such material could be standardized for one series of animals only by using for each animal of that series the material from the same mixture. The amount of the antigen was constant and in the first set of experiments only the quality of the antigen varied. Living and heat-killed colon bacilli were used.

IMMUNIZATION WITH LIVING AND HEAT-KILLED COLON BACILLI

In order to test the difference of the antigenic value of heat-killed and living colon bacilli (culture no. 300), two sets of animals

TABLE 2

Immunization with Living and Heat Killed Colon Bacilli Followed by Colon Bacillus-Gum Tragacanth Peritonitis

DOGS	TYPE OF VACCINE	DAYS BETWEEN FIRST IMMUNIZING INJECTION AND PERITONITIS	DOGS SURVIVED	ANIMALS SURVIVING
				per cent
10	Living colon bacilli	10	10	100
10	Heat killed colon bacilli	10	10	100

consisting of ten dogs each were used. One set was immunized on four successive days by the intraperitoneal introduction of one, two, three and four billion living organisms respectively. The other set received heat-killed *Esch. coli* (one hour at 56–60°C.). Ten days after the first immunizing dose, both sets of dogs with five control animals were injected with three billion living bacteria suspended in 40 cc. of 2.5 per cent gum tragacanth. The control animals died within twenty-four hours with a severe hemorrhagic-fibrino-purulent peritonitis. All the animals of both sets survived (table 2).

Two other sets of dogs were employed to test the relative efficacy of living and heat-killed *Esch. coli* in protecting the perito-

neum against fecal peritonitis. One set of twenty animals was immunized intraperitoneally with living colon bacilli, another set of ten animals with heat-killed bacteria, in a manner similar to the first two sets. Ten days after the first immunizing injection, the thirty animals with five control dogs were injected intraperitoneally with a mixture of fecal material in saline as described above. The five control dogs died in from twelve to thirty-six hours with a marked fibrino-purulent peritonitis. One dog out of the twenty immunized with living organisms died. Four out of the ten immunized with heat-killed bacteria died (table 3).

TABLE 3

DIFFERENCE IN THE PROTECTION CONFERRED BY IMMUNIZATION WITH LIVING AND HEAT KILLED COLON BACILLI IN FECAL PERITONITIS

DOGS	TYPE OF VACCINE	DAYS BETWEEN FIRST IMMUNIZING INJECTION AND PERITONITIS	DOGS SURVIVED	ANIMALS SURVIVING
				per cent
20	Living colon bacilli	10	19	95
10	Heat killed colon bacilli	10	4	40

COMMENT

It is apparent that at least in one set of experiments, heat-killed colon bacilli were able to confer immunity against the homologous organism in a degree equal to that achieved by living bacteria. The colon bacillus-gum tragacanth peritonitis which was induced represents an infection several times the lethal amount necessary to kill a dog of 10 kilograms in weight.

The results of the second experiment in which the dogs were given fecal peritonitis may be interpreted as follows: the fecal peritonitis produced was of a more severe grade than the colon bacillus infection and the heat-killed vaccine was not so efficient an antigen as the living bacteria. It may be concluded also that the infection produced and the immunity achieved bear a quantitative relationship to each other. The immune response as repre-

sented by the number of cells and the rapidity with which they appear must be at least equal to the degree of infection. The fecal material injected contained many different bacterial species. Since the immunizing agent consisted of a single organism and the invading bacteria of several, it may be assumed that the immunity developed was at least in greater part of a nonspecific nature.

INTRAPERITONEAL IMMUNIZATION WITH HEAT-KILLED COLON BACILLI AND PRODUCTION OF A COLON BACILLUS PERITONITIS AFTER VARYING INTERVALS

In previous communications,[9, 10] it was pointed out that when peritonitis was produced a day after the intraperitoneal introduction of a bacterial vaccine, the peritoneal protection obtained was due to a coincident presence of phagocytes. The serum and the

TABLE 4

Intraperitoneal Immunization with Heat Killed Esch. coli and Production of Esch. coli Peritonitis after Varying Intervals

DOGS	DAYS AFTER THE LAST IMMUNIZING INJECTION	DOGS SURVIVING
		per cent
28	1	65
10	14	100

peritoneal exudate were found not to contain any humoral antibodies. The following experiment was performed to evaluate, at least in on approximate relative quantitative measure, the part played by the already present peritoneal cellular exudate and the active immune humoral and cellular processes. Thirty-eight dogs were given four intraperitoneal injections of heat-killed colon bacilli on four successive days. In twenty-eight of these animals, a colon bacillus-gum tragacanth peritonitis was produced the day following the last protecting injection. The remaining ten animals were given a similar peritonitis but fourteen days after the first protecting dose. Peritoneal cell counts taken on the fourteenth day after the first vaccine injection revealed the presence of an average number of 116,000 white cells per cubic centimeter in the peritoneal cavity. Of these cells the polymorphonuclears

constituted 72 per cent and monocytes and clasmatocytes the other 28 per cent. Of the twenty-eight dogs there was a survival of 65 per cent of the animals; of the ten dogs 100 per cent survived. Within the limits of this experiment it may be assumed that the active immunity process accounts for 35 per cent of the survivals or if the protective process were considered on the basis of 100 per cent, the active immunity is equivalent to 35 per cent and the coincident phagocytosis by the leukocytes present in the peritoneal cavity is responsible for 65 per cent of the protection conferred (table 4).

RELATIVE PERITONEAL CELL AND BACTERIAL COUNTS IN NORMAL AND PROTECTED ANIMALS WITH COLON BACILLUS PERITONITIS

Since most of the unprotected control dogs died within the first twelve hours following the production of a colon bacillus-gum tragacanth peritonitis, it was assumed that the survival or the death of the animal was decided within those hours. After peritonitis was induced in animals, peritoneal and bacterial counts were done hourly for eleven hours on control dogs, and in vaccinated animals with peritonitis the day following the last vaccinating dose and fourteen days after the first protecting injection. The relative curves of the peritoneal cell counts are shown in the accompanying chart. The cell counts of the control animal were the lowest of the three. The largest number of cells was present in the dog with peritonitis one day following the last protective injection. It is apparent that in the animal with a definitely established immunity, the cellular response is less than in a dog without such an immunity. However, the previous experiment demonstrated that the actively immune animal possesses approximately 35 per cent greater protection. Under the conditions of these experiments, it may be inferred that the difference in the protection is due to the presence of humoral antibodies which are either bactericidal in themselves or assist the cells in bacterial destruction.

The bacterial counts of the peritoneal fluid were performed by the method of dilution and plating. Hence, all the viable bacteria whether free or phagocytosed were accounted for in the counts.
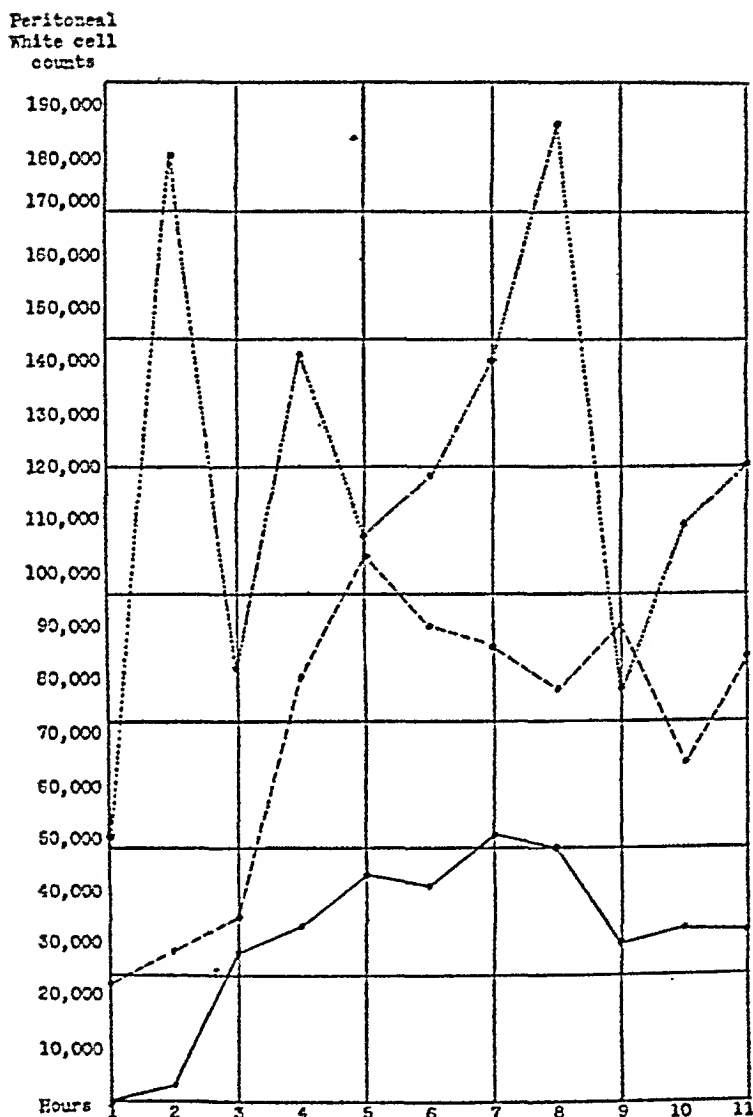
CHART 1. PERITONEAL WHITE CELL COUNTS PER C.M. OF PERITONEAL FLUID

The solid line indicates counts on a normal dog, the line of dashes, a dog with colon bacillus peritonitis fourteen days after the first immunizing injection of vaccine, and the line of dots, a dog with colon bacillus peritonitis on day following the last injection of vaccine.

The relative counts in table 5 reveal that in the control dog with peritonitis, the bacteria persist in large numbers (millions) in the peritoneal exudate; the dog with peritonitis the day following the last vaccination possessed the smallest number of viable bacteria (few thousands). Smears of the peritoneal exudate disclosed that these viable bacteria were within phagocytes. In the animal with a definitely established active immunity, the bacterial destruction was not so rapid nor as thorough as in the dog protected one day. The difference is probably accounted for by the smaller number of

TABLE 5

RELATIVE PERITONEAL BACTERIAL COUNTS IN A CONTROL DOG AND VACCINATED DOGS WITH COLON BACILLUS PERITONITIS

HOURS AFTER ONSET OF PERITONITIS	BACTERIA PER CUBIC CENTIMETER OF PERITONEAL FLUID		
	Control dog	Dog with peritonitis one day following last protecting injection	Dog with peritonitis 14 days after the first protecting injection
1	27,360,000	20,000	18,400,000
2	33,000,000	160,000	2,360,000
3	14,000,000	No growth	400,000
4	6,740,000	No growth	640,000
5	880,000	40,000	480,000
6	240,000	12,600	520,000
7	560,000	9,400	80,000
8	14,000,000	8,400	82,000
9	10,300,000	5,800	20,800
10	4,160 000	4,000	80,400
11	Dead	5,600	15,400

phagocytes in the peritoneal exudate (see chart). These experiments allow the conclusion that the animal protected one day disposes of the offending bacteria more efficiently and rapidly. This bacterial disposal is due entirely to phagocytosis as seen by the absence of demonstrable humoral antibodies and by the observation of phagocytosis in the peritoneal smears. It can be again inferred that other factors than phagocytosis play a part in the protection of the actively immunized animal. It is assumed that these other factors are probably humoral antibodies.

IMMUNIZATION BY THE SUBCUTANEOUS ROUTE

Since the experiments pointed to the establishment of an active immunity, general in character but with a local manifestation it was assumed that subcutaneous immunization should produce at least partial immunity.   For the following experiment, two sets of dogs were used.   Four daily subcutaneous injections of living *Esch. coli* of one, two, three and four billion organisms were made in a set of six dogs.   Another set of nine animals received living *Esch. coli* intraperitoneally on four successive days.   Fourteen days after the last immunizing injection both sets of dogs and four control animals were given *Esch. coli*-gum tragacanth peritonitis. The four control dogs died within twelve hours with a severe hemorrhagic-fibrino-purulent peritonitis.   The two sets of dogs, six and nine in number, survived.   Apparently, the general immunity established by the subcutaneous route was sufficient to protect the animals from an otherwise lethal peritonitis.

SUMMARY

Heat-killed colon bacilli used as intraperitoneal vaccine can induce a protection against a colon bacillus peritonitis equal to that of living bacteria.   However, in fecal peritonitis the living colon bacilli confer a greater protection than heat-killed organisms. It is assumed that this difference is due to a greater antigenic value of living bacteria.   Because colon bacilli vaccinated animals survive fecal peritonitis, (feces contain many species of bacteria) the immunity is considered, at least in greater part, non-specific.   The protection against acute diffuse peritonitis is of a two-fold nature; a general immunity and a coincident presence of phagocytes in the peritoneal cavity with a consequent phagocytosis.   The active general immunity can be evaluated as 35 per cent and the local phagocytosis as 65 per cent under the conditions of these experiments.   The greater number of these phagocytes is polymorphonuclear in type.   This general immunity is apparently sufficient to protect animals by subcutaneous route against a colon bacillus peritonitis.

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THE RELATIVE VALUE OF CULTURAL METHODS AND GUINEA PIG INOCULATION IN THE DIAGNOSIS OF TUBERCULOSIS*

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In recent years a lengthy discussion has been carried on relative to the value of cultural methods and use of guinea pigs in the diagnosis of tuberculosis. It has led to a rather sharp division of opinion which has obscured the facts and resulted in individuals advocating one method to the exclusion of the other. With a view of carefully appraising the methods, we have reviewed the work of previous authors and have conducted extensive experiments of our own. Advantages and disadvantages have been found in both methods and wise clinical pathologists will make use of each for what it does best. Out of the controversy has certainly come improved and excellent methods for culturing organisms of tuberculosis, stimulated by Löwenstein[23] in Europe and Corper[5] in America. Furthermore, a clearer understanding of the limitations of animal inoculation has been realized, as well as the great value of testing clinical material by inoculation of guinea pigs.

SPONTANEOUS TUBERCULOSIS IN GUINEA PIGS

One of the objections that has been made to the use of guinea pigs in the diagnosis of tuberculosis, is that these animals, since they are susceptible to the disease, may become spontaneously infected, giving rise to a false positive. Robert Koch[22] was the first to issue a caution concerning the interpretation of results in guinea pigs. Although he never had a tuberculous pig brought

to him for study, he did report on seventeen that contracted tuberculosis while under observation in the laboratory in which other tuberculous animals were kept, and he stated that caution should be exercised in the interpretation of results when guinea pigs are kept for more than three months in the room with tuberculous animals.

Calmette[2] who has had a vast experience with guinea pigs, stated that tuberculosis practically never affects them under ordinary circumstances. This same opinion had previously been voiced by Corbett.[4] Guinea pigs can, however, be readily inoculated by nasal spray, and Römer and Joseph[38] maintained that the guinea pig is so susceptible to tuberculosis that one single bacillus probably can produce tuberculosis in them.

Bartel[1] found that suckling guinea pigs became tuberculous if the mother had the disease. The investigation of Remlinger disclosed that even though guinea pigs were confined with other animals which had cutaneous ulcers due to tuberculosis, very few would develop the disease; thus, of forty-three normal guinea pigs confined with those which had cutaneous ulcers, until the latter died, tuberculosis developed in only three. Fifty-two guinea pigs were fed for a year on material which had been soiled by tuberculous animals, without contracting the disease. Sewall and Lurie[42] confined guinea pigs under small bell jars used for both positive and negative animals, and returned these animals to crowded, unsterilized cages; a third of eighteen studied contracted the disease.

Perla[34, 35] demonstrated that after intraperitoneal inoculation, *Mycobacterium tuberculosis* is eliminated in the feces for the first week, following which none appears for several weeks unless the disease is widely disseminated in the body. In such instances organisms occasionally appear in the bile and in the urine. He quoted Koch to the effect that spontaneous tuberculosis in animals was in direct proportion to the number of animals kept in the room, and that the mode of transmission was respiratory. Perla conducted experiments to demonstrate the effect of overcrowding and the presence of tuberculous cutaneous ulcers and their importance on the development of spontaneous tuberculosis

in guinea pigs. Under the conditions of the experiment a few of these guinea pigs contracted tuberculosis, and when normal pigs were confined in the same cages with infected animals the portal of entry was by the intestinal tract. He also concluded that when guinea pigs were kept in the same room with tuberculous animals even though they were in different cages, after three months they were not suitable for experimental purposes; in such instances the portal of entry was the respiratory tract.

Griffith[17] observed seven cases of spontaneous tuberculosis in guinea pigs, over a period of fifteen years. He stated that in 1928 he had two in which the infection proved to be avian in type. In 1927 he observed another case due to the human organism of tuberculosis. Unfortunately, these animals had been used previously for injection of tuberculous material, and this would cast some doubt on the reliability of the observation.

Reisman and Baylis[37] performed, the following experiment: twelve young, presumably normal guinea pigs were allowed to remain for four days in an enclosure on the floor of the animal room; then, a second lot of animals was placed in the same enclosure. At the end of the period, 75 per cent of the first lot gave positive skin reactions with tuberculosis and 25 per cent of the second. Some of the guinea pigs were subjected to necropsy; in only two were acid-fast bacilli found, but these organisms were not tested for virulence. The authors concluded that tuberculosis is quite prevalent in apparently normal guinea pigs. The conclusion is especially interesting in view of the work by Thompson and Frobisher,[47] who quoted Corper and Petroff to the effect that they found acid-fast bacilli in 36 per cent of a large series of animals previously given injections of filtered tuberculous material, but that they also demonstrated such organisms in 33 per cent of normal animals which had not been given injections. Thompson and Frobisher performed a similar experiment with similar results, and stated that all observers failed to culture or identify the organism; hence, they felt that the presence of acid-fast bacilli in the lymph nodes of guinea pigs should not be taken as an indication of tuberculosis.

Sewall[41] is of the opinion that guinea pigs kept in sanitariums for

tuberculosis should be very carefully guarded against being given scraps from the tables of patients and protected from contact with caretakers who might have tuberculosis.   Lurie,[24,25,26,27] in a series of experiments on the experimental epidemiology of tuberculosis, has concluded that the more guinea pigs are crowded the more spontaneous tuberculosis appears in them.   However, as an index of air-borne infection, he was able to keep twelve controls in the room, not in contact with the others,—negative during the year of observation.   In a room in which the number of tuberculous guinea pigs varied, out of 103 which originally were free of tuberculosis and which were exposed for thirty-two months, 14.5 per cent developed tuberculosis; obviously the respiratory tract was the portal.   Even when normal guinea pigs were exposed in the same cages with others which had tuberculosis, and the cages were made with wire mesh bottoms, practically all of the evidence pointed to the fact that the infection was pulmonary, and was not developed until after an average of 293 days.   In an experiment he controlled conditions to some extent, so that guinea pigs could acquire tuberculosis either by the respiratory or the digestive tract.   He was able to show that when guinea pigs acquired tuberculosis naturally in this fashion, the infection did not become disseminated, but remained near the portal of entry.

In order to test to some extent the question of spontaneous tuberculosis occurring in guinea pigs, a series of observations and experiments was made in our laboratory.   Guinea pigs have been used as experimental animals in The Mayo Clinic for about twenty-seven years; during this time thousands of these animals have been subjected to necropsy.   Although specific search for tuberculosis has not been made in all cases, nevertheless records are available which include more than 25,000 postmortem examinations of guinea pigs which have been received from scores of different dealers in the United States, in particular from those in the middle west.   In no instance has spontaneous tuberculosis been encountered in one of these animals.   In addition to this evidence, one of us (T. B. M.) has given injections to more than 15,000 guinea pigs for diagnosis of tuberculosis, all of which have been subjected to careful necropsy.   Approximately 15 per cent

of these animals has been positive, which means that a positive diagnosis has been made on about 1,200 specimens by this method. In most instances it has been possible to confirm the findings by operation on, extensive clinical observation of, or necropsy of the patient, and in not a single instance has a false positive been reported, which indicates even more strikingly the fact that spontaneous tuberculosis in animals which have been brought to the laboratory for use has not occurred in our series.

However, the question of whether animals can contract tuberculosis when kept in the same room or the same cages with animals which have tuberculosis is quite a different problem, and has an important bearing on how animals are to be cared for during experimental work. Accordingly, a series of experiments was designed to test the development of spontaneous tuberculosis in guinea pigs in our laboratories under certain conditions.

The animal room, which is used exclusively for housing animals that are to be used in tests for tuberculosis, is 20 feet wide and 40 feet long. Cages are arranged in four groups, with forty-eight cages in each group. The individual cages, of all-metal construction, are designed to house two guinea pigs each, and to allow 108 square inches to the pig. There is provided a steam sterilizer for sterilizing the cages after each pair of guinea pigs has occupied it.

Four cages were selected, one from each group of cages, so chosen that two were on the bottom row and two on the top row. Into each of these cages two normal guinea pigs were introduced. They were cared for by the same caretaker who handled the rest of the animals in the room.

In the first cage one animal died at the end of fifteen days, and the other one was killed at the end of 396 days. Neither animal showed any signs of tuberculosis. In the second cage, one animal lived 137 days and the other was killed at the end of 396 days. Neither animal showed any signs of tuberculosis. In the third cage one animal died at the end of thirty-four days, the other one at the end of seventy days, neither one showing any signs of tuberculosis. In the fourth cage, which was one on the bottom row of cages, one animal was killed at the end of 396 days, showing no signs of tuberculosis, whereas the other animal of the pair died in 221 days with typical tuberculous lesions in the liver, spleen, lungs and bronchial lymph nodes.

For other experiments special cages were designed, of such size that each guinea pig was allowed fifty-two square inches of space. These cages were open at the top and were arranged in the following fashion: Cage 1, beginning on the left, was a cage in which were placed ten normal guinea pigs. Cage 2 was separated from number 1 by half inch mesh wire screen, so that it was possible

for portions of food and bedding to be interchanged between the cages by the antics of the guinea pigs. In this cage were placed ten guinea pigs, five of which had been given intraperitoneal injection, and five subcutaneous injection of an emulsion of tuberculous spleen, freshly removed under precautions of sterility, from a guinea pig. In this same cage were placed fifteen normal guinea pigs. Cages 3 and 4 were duplicates of cages 1 and 2, both in size and in the manner in which the experiments were carried out. The same person who took care of the other guinea pigs in the room took care of cages 2 and 4, whereas another person, who had nothing to do with tuberculous animals, took care of cages 1 and 3. The cages were cleared of bedding once a week and only one supply of water and one hopper of dry food was kept in each cage.

Of the guinea pigs in cage 1, four were dead by the end of thirty-eight days; the other six lived from fifty-six to 396 days. None of these animals showed evidence of tuberculosis; apparently they died of various other causes. Of the guinea pigs in cage 2, that received injections of tuberculous material, five died within the first nineteen days and showed no signs of tuberculosis. The other five lived for from forty to eighty-six days, and all had typical miliary tuberculosis. In that cage of the pigs which were not inoculated, eight died within thirty-three days, seven lived for from forty to 396 days; none showed any signs of tuberculosis. In the third cage, which contained pigs which were not inoculated, four died within thirty-seven days; the remaining six lived for from 186 to 396 days and none showed any signs of tuberculosis. Of the inoculated pigs which were kept in cage 4, four died within fourteen days without signs of tuberculosis. The remaining six lived for from thirty-two to 134 days, and showed evidence of typical miliary tuberculosis. Of the fifteen pigs which were not inoculated but which were housed in the same cage, eight died within thirty-two days and the remaining seven died in from ninety-two to 396 days; all of this lot were negative for tuberculosis except one pig, which lived 179 days, and which, at necropsy, revealed a typical tuberculous spleen.

It is interesting to note that four young guinea pigs were born to mothers in the second cage; one of them died 240 days later, two were killed 300 days later, and a fourth died 330 days later. Three additional animals were born in the same cage and lived about ninety days each. None of these members of the second generation showed any signs of tuberculosis.

From those observations and experiments, and from the observations of others, it becomes evident that when guinea pigs are kept in cages in a room free from tuberculous animals, and receive no food from the tables of tuberculous patients, and are not cared for by tuberculous caretakers, the chances of their becoming infected with tuberculosis may be totally ignored, and any such animal, provided it is in normal health, may be considered suitable for experiments on tuberculosis.

It is possible for animals, especially if they are over-crowded when kept in the same room with tuberculous guinea pigs or other tuberculous animals to contract spontaneous tuberculosis. Under the worst conditions, in only a relatively few animals will such lesions develop and these will not develop in less than ninety days and usually a longer time will be required. When such lesions occur they are usually discrete and can usually be distinguished from the lesions produced by experimental inoculation of the animals, as has been shown by numerous workers. It is evident that under most conditions, by use of very simple precautions, stock guinea pigs could be kept in the same room with a few animals which were used for the diagnosis of tuberculosis. If one were using only a few such animals, very little exposure would ever be brought to the normal stock guinea pig. If many animals are used, such a laboratory would undoubtedly have available a stock-room which would be different from the room in which the diagnostic pigs were kept, and under these conditions spontaneous tuberculosis in experimental guinea pigs would be effectively ruled out.

CULTURE METHODS FOR MYCOBACTERIUM TUBERCULOSIS

It is not the purpose of this paper to review in detail the various methods which have been proposed for culturing *Mycobacterium tuberculosis*. However, it is desirable to give a résumé of the important steps which have been taken in bringing this useful laboratory method to its present state. Robert Koch[22] successfully grew bacilli of tuberculosis in 1882, using coagulated blood serum derived from tuberculous tissue. While some improvement was made after that time, it was not until Petroff, in 1915, devised his method that the culturing of bacilli of tuberculosis from clinical material became at all practical. To be sure, Uhlenhuth had introduced antiformin as an anticontaminant reagent, but Petroff introduced 3 per cent sodium hydroxide as a killing agent for nonacid-fast bacteria, which was far superior. He made use of egg medium of Dorset, which had been successfully used by that author in 1902, and added to it besides certain other ingredients and gentian violet to suppress the growth of

gram-positive cocci.   Between the time of Koch's and of Petroff's work, Roux and Nocard had discovered the importance of glycerol in mediums used for growing bacilli of tuberculosis.   Although Petroff's method has been extremely useful, it has by no means proved a satisfactory medium for the routine culturing of bacilli of tuberculosis, derived from clinical material, and the introduction of the combination of killing nonacid-fast bacilli with sulphuric acid, and the subsequent planting of the sediment on glycerol-potato medium was introduced by Sumiyoshi[44] in February, 1924.   This was followed, the next month, by further studies by Löwenstein[23] using this method.   Hohn,[20] in 1926, modified the Sumiyoshi-Löwenstein method in two particulars: by the use of the unwashed sediment for culturing, and by the substitution of egg medium for glycerol-potato.

In the hands of these authors and numerous others, these methods have proved practical and successful, and it has been demonstrated beyond any question that positive cultures often can be obtained when specimens are microscopically negative. Corper[5] has estimated that unless 100,000 bacilli are present in 1 cc. of sputum, it is not possible to detect them in the stained smear; however, Pottinger[36] claimed, by his method, to be able to demonstrate bacilli of tuberculosis when they were no more numerous than 250 for each cubic centimeter.

Corper   and   Uyei[6, 7, 8, 9, 10, 11]   have   studied   the   Sumiyoshi-Löwenstein method extensively, and have introduced several modifications; using potato cylinders, first devised by Pawlowsky,[33] they added crystal violet and glycerol water, treating contaminated material with oxalic acid, 5 per cent, which, after a long series of experiments, they concluded was the best method for culturing *Mycobacterium tuberculosis*.   So successful were they with this method, that they were able to grow bacilli of tuberculosis from 91.4 per cent of sputums demonstrated microscopically to have bacilli in them.   Matthies[29] reported that using Hohn's modification, cultures were obtained in nearly all material which was demonstrated to be microscopically positive.

Sweany and Evanoff[45, 46] have proposed using a medium composed of veal, milk, cream, and egg, using 5 per cent hydrochloric

acid or 3 per cent sodium hydroxide for killing nonacid-fast bacilli. This medium has been slightly modified by Feldman,[14] and has proved extremely satisfactory in isolating bacilli of tuberculosis, and in particular the bovine type. Still more recently, Miraglia[31] has introduced glycerol-broth-egg-yolk medium, which in his hands and in the hands of Feldman,[15] has been extremely satisfactory, in particular for the human type of *Mycobacterium tuberculosis*. Still more recently, Herrold[18] has used egg-yolk in nutrient agar, pouring plates or slants. He concluded that this method was more certain than smears and is as delicate and more prompt than guinea pigs. Strangely enough, however, the author made these sweeping conclusions on the basis of few specimens. There were three specimens of urine, two of which were shown to contain bacilli of tuberculosis by smears, and all gave growth in from seven to ten days; three specimens of sputum all of which were shown to contain bacilli of tuberculosis by smear, gave growth at the end of ten days; one specimen of spinal fluid, the smears of which were negative, gave growth on the tenth day, and two specimens of thoracic fluid, smears of which were negative, gave colonies in six days. So far as can be determined from Herrold's paper, guinea pigs were used only in a dilution experiment, in which five guinea pigs were used for comparison of results with those obtained by examination of smears and by culture. The guinea pigs and the cultures ran parallel; the smears became negative in the high dilutions.

As the matter now stands, it may be conceded without any question that bacilli of tuberculosis can be cultivated readily in a high percentage of instances when they can be found in direct smears, and that colonies will appear so that they can be detected after a period of ten days or more, average two to five weeks; the time depends somewhat on the medium used, the method of destroying contaminants, the type and number of bacilli in the material. As compared with the method of direct staining, it is evidently more sensitive, but enough evidence is not available to give a fair estimate as to how much more sensitive it actually is, and indeed the problem hardly seems to warrant the time and money it would take to solve it.

Although it is one thing to demonstrate the presence of bacilli of tuberculosis by cultural methods in material which on microscopic examination can be shown to contain these organisms, it is quite another to culture organisms from material which is microscopically negative. Since from a diagnostic standpoint this is the most important phase of the study, we have made a thorough comparison between the results of such cultures and inoculations of guinea pigs.

CULTURES COMPARED WITH ANIMAL INOCULATION

Löwenstein[23] studied fourteen specimens of urinary sediment, which were cultured and inoculated into guinea pigs. Tuberculosis in three animals failed to develop, but the cultures were positive. Löwenstein did not present any evidence to indicate whether these cultures were of virulent organisms, or whether the patients from whom this material was collected had tuberculosis.

Clairmont[3] found in the examination of clinical material one instance in which culture was positive and the animal negative, whereas three times the animal was positive and the culture negative. Information is not available as to the exact interpretation of these results.

Seelemann and Klingmüller,[40] as a result of experiences with Hohn's procedure, came to the conclusion that inoculation of animals is to be preferred on account of its greater certainty, especially when the material is contaminated with spore-forming microörganisms.

Corper,[5] and Corper and Uyei,[8] who advocated the cultural method as a certified diagnosis for tuberculosis, came to the conclusion that this method is equal to inoculation of guinea pigs and has many advantages. The basis on which this conclusion is drawn, however, will bear some examination. For instance, they studied ninety-three microscopically positive specimens of sputum. They obtained positive cultures in 91.4 per cent, but made the following interesting and suggestive statement: "To have done guinea pig inoculation tests in this series would obviously have been unnecessary so they were omitted." In a series of nine

microscopically doubtful specimens of sputum, the guinea pig and culture method ran parallel. Of twenty-four specimens of urine these investigators used, three gave positive cultures by Petroff's method, ten gave growth in potato medium, and eleven gave positive results with guinea pigs. The tissues from dogs and rabbits infected with tuberculosis, when cultured and also used for inoculation of guinea pigs, gave results about parallel, but the nature of the experiment is such that it is difficult to point out advantages for either method. The same experimenters stated that the cultural method would usually give positive results in the fourth to the fifth week, and guinea pigs in the third to the fifth week. They further stated that there is no difficulty in determining pathogenic from nonpathogenic acid-fast bacilli, since the usual nonpathogens will grow on the ordinary culture mediums in from two to five days. Apparently they did not do virulence tests on the organisms isolated, nor did they correlate any of their results with the condition of the patients.

Corper and Uyei,[11] in comparing various methods of growing bacilli of tuberculosis, have reported in their tables on the basis of the number of tubes inoculated, from which they obtained percentages of positive growth. Interpreting these results on the basis of clinical specimens is not altogether obvious, unless one has available the exact protocols of each group of cultures. Very different percentages might be obtained on the basis of the number of specimens used from those on the basis of the number of tubes used.

The difficulty of obtaining growth of bacilli of bovine tuberculosis on mediums which have been devised by Corper has been reported by Feldman,[15] who was able to show that the medium of Sweany and Evanoff was superior in this regard. Even Corper and Uyei[11] had difficulty in obtaining a large percentage of positive cultures with bovine material when compared with the percentage of positive results obtained with inoculation of guinea pigs. Thus, of sixteen specimens used, nine proved positive by cultural methods, whereas all specimens were positive, judged by results of inoculation of guinea pigs. The importance of this in relation to the diagnosis of tuberculosis in man is obvious, when

one recalls the rather high percentage of bovine tuberculosis in human beings that has been reported from time to time by various authors, and the more recent report of Van Es and Martin,[45] who obtained bovine tuberculosis in several specimens derived from human beings, including those sent to them from The Mayo Clinic.

Herrold's[18] work has been considered elsewhere in the paper; suffice it to say that on a basis of very little evidence he concluded that cultures were more delicate and results of cultures more prompt than inoculation of guinea pigs.   Nevertheless, Woolsey,[49] has submitted the medium to a more critical test.   She made a routine of testing suspicious fluids, both by this cultural method, slightly modified, and by inoculation of guinea pigs.   Of 130 cases, in twenty-five cultures were positive and in twenty-three results with guinea pigs were positive; there was agreement in 121 instances.   The cultures became positive, on an average, in eleven days, whereas the diagnosis by inoculation of guinea pigs was not made until six weeks had elapsed.   As Woolsey became more proficient with the medium, her cultures became positive, on an average, in five days; some in as short a time as two days.   In her experiments with animals she used the inguinal nodes, making smears and sections of them, without depending on gross characteristics.   Of five of the nine cases in which the results disagreed she concluded that the Herrold medium was the more reliable. Three of her cultures became over grown and had to be discarded. She injected the isolated bacteria into guinea pigs when there was disagreement between the results of culture and the original inoculation of guinea pigs.   By the use of guinea pigs, three cases were detected in which the cultures were negative, whereas by the use of cultures four cases were identified in which the guinea pigs were negative.

Hüth and Lieberthal,[21] reporting on 1,200 cultures made by Hohn's method, stated that this method is to be preferred to the use of guinea pigs.   Protocols were omitted from the paper, and it is evident that the same material was cultured and also inoculated into guinea pigs in only "about ten cases;" yet the authors used the expression "in many cases in this latter group" referring

to the cases in which guinea pigs were inoculated, and they further stated that: "because of its absolute reliability and simplicity the culture method is of greater value than the guinea pig inoculation." From the material they presented, it is impossible to know on what basis these conclusions were drawn.

Schmidt[39] examined forty specimens of sputum in which bacilli of tuberculosis were scantily present. When cultured, they gave, with the egg medium, six positives, and with the potato medium, five, whereas eleven guinea pigs gave positive results. Schmidt concluded that the animal test was more sensitive than the culture method. In one case, however, the animal test was negative and the culture positive, so that he recommended using both methods.

Dimtza,[12] studying specimens obtained at surgical operation, compared the results of culturing with inoculation of guinea pigs. He used a slight modification of Hohn's method but did not give the method used in handling the animals. He examined 500 specimens, of which he concluded 126 were tuberculous. Of these, 104 (83 per cent) proved to be positive by direct smear, 117 (93 per cent) were demonstrated to be positive by inoculation of animals, and 122 (97 per cent) were positive by culture. In one case direct smear was positive, and the other two methods gave negative results. In three cases both smear and culture were negative, and in eight both smear and inoculation of guinea pigs gave negative results. No evidence was presented to prove that the eight positive cultures were actually *Mycobacterium tuberculosis*, since he apparently did not test them in animals. Obviously only eleven specimens that were microscopically negative were tested, and, lacking the protocols of the eight negative animals, final conclusions are not evident.

Recommendations similar to those of Schmidt were made by Sweany and Evanoff.[45] They found agreement in direct smears, cultures, and inoculations of animals in study of ten specimens of material suspected of being tuberculous, and in which all three methods gave negative results. With fourteen specimens the following results were obtained: three specimens were positive by direct smear, culture, and animal inoculation; the other eleven

were negative by direct smear.   Of these eleven, nine were positive both by culture and inoculation of animals.   Two were negative by cultural methods and positive by inoculation of animals, and three were negative by inoculation of animals and positive by culture.   These three cultures were injected into guinea pigs and proved to be bacilli of tuberculosis.   The number of animals given injections of each specimen was not given, but the authors stated that there were 11.7 per cent more positive results on culture than on inoculation of animals.   It is not at all evident how these figures were obtained, since out of a total of fourteen specimens, eleven were positive by animal inoculation and twelve were positive by cultural methods.   The authors concluded that for diagnostic work both methods should be used.

Stadnichenko and Sweany[43] later compared inoculation of guinea pigs and cultures in a series of 200 specimens of sputum of selected patients.   One hundred thirty-one patients were found to be negative as judged both by results of inoculation of guinea pigs and of culture; thirty-three were found to be positive as judged by results of both methods.   In thirty-three cases inoculation of guinea pigs gave positive results whereas the cultures were negative, and in three cases the cultures were positive whereas guinea pigs were negative.   Two of these cultures were tested on animals and proved to be virulent.   They concluded that inoculation of guinea pigs was superior to the cultural method for badly contaminated material such as sputum.   Cultural studies had the advantage of being more absolute, less expensive and of permitting of more repetition.   The authors voiced a warning that atypical acid-fast bacilli should be submitted to animal experimentation before a positive diagnosis was given.   They also expressed the belief that certain specimens will produce growth only when treated by acid; others, only when treated by alkali.

Woolsey and Petrik[50] compared the results of inoculation of guinea pigs with cultures after having devised a potato-egg medium which they claimed to be superior both to Petroff's and to Corper and Uyei's medium.   The material tested was specimens of sputum from patients either known to have tuberculosis or

suspected of having it, and in which only a few bacilli, or none at all, could be found. They concluded that acid digestion of the sputum resulted in fewer contaminations, but that alkalies made better agents of digestion. Comparing the results of inoculation of guinea pigs with results with their own medium it became quite evident that a higher percentage of positive tests could be obtained by use of guinea pigs than could be obtained by culturing. The authors pointed out that one can inoculate guinea pigs with larger amounts than it is practical to use in cultures, and that this often accounts for the higher percentage of positive results with guinea pigs. Trying this out on a quantitative basis they concluded that at least twenty tubes of this medium would have to be inoculated in order to equal the accuracy of inoculation of guinea pigs when the animal receives a considerable amount of material. Nevertheless, they claimed that positive results can be obtained more readily and earlier by the cultural method. The earliest growth was obtained after eleven days, and the latest after fifty-six days, with an average of twenty-one days. Fifteen per cent of all tubes were contaminated, but no set of tubes was completely contaminated. The guinea pigs were tested with tuberculin once a week, and as soon as this test was positive, they were killed. The earliest was killed in eighteen days after inoculation; the latest, in sixty-eight days, and the average, in thirty days. No animals were called positive unless the organisms were demonstrated in the lymph nodes.

In a personal communication from Hillkowitz,[19] he stated that Puntoni summed up the whole matter as follows: "The cultural method, according to the most recent research, cannot presume to be a substitute for the guinea pig inoculation, which always gives the greatest number of positives. Nevertheless, it presents certain advantages especially speed and occasionally positive results when the biologic test fails."

As a result of about fifteen years' experience with inoculation of guinea pigs for diagnosis of tuberculosis, and on the basis of carefully controlled tests, we[16] have found that the safest procedure for inoculation of animals with clinical material is as follows: the material is centrifuged for one hour at 2,500 revolutions per

minute, in a centrifuge that has a head radium of 13.5 cm. At the end of this time the top 1 cc. is pipetted off, and after the middle portion has been discarded, this top 1 cc. is added to the sediment. The whole is now made up to 5 cc. with physiologic saline solution, and two guinea pigs are given injections, one subcutaneously and one intraperitoneally. Guinea pigs which die and show no gross lesions by the end of three weeks are considered failures; if no lesions are present in guinea pigs that die after three weeks, the test is considered negative. Guinea pigs are premitted to live for eight weeks; at the end of that time they are killed, and considered either positive or negative, depending on whether lesions are present or absent. To anyone experienced in the diagnosis of tuberculosis of such animals, gross lesions can be accurately diagnosed without resorting to histologic examination of the tissue. In certain rare cases, especially when the animal has died early, and histologic diagnosis of tuberculosis must be used, it should be based primarily on finding acid-fast bacilli within the lesions.

In an attempt to hasten the diagnosis of tuberculosis in guinea pigs, we[28] experimented with intracerebral inoculation of animals with clinical material. This proved to be unsatisfactory as a routine, on account of the large number of failures among the inoculated animals. In certain instances, however, the diagnosis of tuberculosis in these animals could be made as early as nine days after inoculation.

Some workers have increased the speed of the test by submitting guinea pigs, at the end of three or four weeks, to a test with tuberculin, killing those that give a positive reaction. Our experience with this procedure is too limited to allow a definite opinion to be expressed, but evidence would point to the fact that in the majority of cases this procedure is safe.

EXPERIMENTAL

In comparing results of cultures of clinical material with inoculations of guinea pigs, approximately one-third of the material was seeded in not less than eight tubes of culture mediums, whereas the rest of it was inoculated into a pair of guinea pigs, and almost always the animals were allowed to live until they died, or at least fifty-eight days. The mediums used were those proposed by

Corper and Uyei, Sweany and Evanoff, and Miraglia. Not all of the specimens were cultured by all three methods, but most of them were. In addition, both

TABLE 1

DAYS ELAPSING BEFORE INOCULATED GUINEA PIGS, AND CULTURES BECAME POSITIVE

(All material microscopically positive)

URINE (21 SPECIMENS)		SPUTUM (10 SPECIMENS)		JOINTS, TISSUES, ETC. (6 SPECIMENS)	
Guinea pigs	Cultures	Guinea pigs	Cultures	Guinea pigs	Cultures
59	45*†	48	17‡	20	63§
27	32§	61	16‡	36	53*
55	48§	48	14‡	62	28*
42	37*	29	20§	42	13§
51	60§	62	10§	22	25*‡
57	30*§	60	15‡	62	13*‡§
56	28†	40	16‡§		
63	35*	61	16‡		
35	37*	60	15‡		
59	56§	65	21§		
67	64§				
37	19‡				
43	14‡				
32	18‡				
42	36§				
51	32*				
47	46‡				
40	Negative	Tuberculosis, bilateral renal (clinical), pulmonary (clinical), knee (surgical pathology)			
Failure	Negative	Tuberculosis, left renal (surgical pathology), pulmonary (clinical)			
Failure	Negative	Tuberculosis, left renal (surgical pathology)			
Negative	Negative	Tuberculosis, left renal (surgical pathology)			
Average..48	38	53	16	41	33

* Glycerol potato (crystal violet).
† Glycerol potato (without crystal violet).
‡ Glycerol-broth-egg-yolk.
§ Veal-milk-cream-egg.

acid and alkali were used as an anticontaminant reagent. Results of the first series of tests are summarized in table 1. All of the material used was demonstrated to contain acid-fast bacilli by direct smear, and the Ziehl-Neelsen stain.

Twenty-one specimens of urine were tested; eighteen produced tuberculosis in guinea pigs, whereas seventeen cultures were positive. Two pairs of guinea pigs died before three weeks had elapsed, and were therefore failures. Cultures from these specimens were negative, and in the remaining case both the cultures and the guinea pigs were negative. The four cases which yielded negative cultures were clearly cases of tuberculosis. The average period of time elapsing before the cultures were positive was thirty-eight days, varying from fourteen to sixty days. The average number of days before the guinea pigs were diagnosed as positive was forty-eight days, varying from twenty-seven to fifty-nine days. Ten specimens of sputum were used, and there was agreement between results of culture and of inoculation of guinea pigs in each instance; all specimens proved to be positive. The average number of days for the cultures to become positive was sixteen, and for the guinea pigs, fifty-three. Six specimens from joints, tissues, and so forth, proved to be positive by inoculation of guinea pigs and culture; the average time for cultures was thirty-three days and for guinea pigs, forty-one days. The greater number of days elapsing before the guinea pigs were positive is partly explained by the fact that the guinea pigs were allowed to live until they died. Diagnosis of many of them could have been made at a much earlier period; one diagnosis was actually made at the end of twenty days.

It becomes evident, therefore, that inoculation of material which is microscopically positive for bacilli of tuberculosis will yield positive cultures in a large percentage of instances, and, by the method of Miraglia, in from ten to thirty days. However, in keeping with the findings of others, in a certain small percentage there is no growth; less often, guinea pigs fail to become tuberculous after injection of such material.

Occasionally, in the routine of work, we have had occasion to inject into guinea pigs material known to contain acid-fast bacilli obtained from lesions which were either then known to be tuberculous or later were demonstrated to be so, and following which tuberculosis failed to develop in the guinea pigs. Two such instances were reported by Morse and Braasch[32] in a series of inoculations of guinea pigs made by one of us (T. B. M.). Although the explanation of this is not altogether apparent, several possibilities suggest themselves: scarcity of bacilli of tuberculosis, the fact that the bacilli may be dead, avirulent or avian, and the fact that bacilli of tuberculosis often appear in clumps, so that those demonstrated on the slide may be the only bacilli present in the material; hence, no organisms might be injected into the

animals. Striking results along this line can be noted in, to get ahead of the story somewhat, table 5; in some animals lesions developed whereas in others none developed, although injections were with material from the same specimen, and were made at the same time.

In order to test the efficacy of cultures as compared with inoculations of guinea pigs, working with clinical material that is negative by direct smear, 100 such specimens, including eighty-four specimens of urine, six of thoracic fluid and ten of material drained from joints, tissues, and so forth, were injected into guinea pigs and cultured simultaneously, using the three methods before indicated.

TABLE 2

Mediums and Anticontaminant Reagents Used for Culture of 100 Clinical Specimens Microscopically Negative*

	CRYSTAL VIOLET-GLYCEROL-POTATO			GLYCEROL-POTATO			VEAL-MILK-CREAM-EGG		
	Specimens	Tubes used	Tubes contaminated	Specimens	Tubes used	Tubes contaminated	Specimens	Tubes used	Tubes contaminated
Sodium hydroxide..	17	103	31 (30%)	8	38	15 (39%)	8	63	57 (90%)
Sulphuric acid.....	26	200	78 (39%)				9	71	36 (50%)
Oxalic acid........	22	158	8 (5%)	10	75	10 (13%)	18	136	81 (60%)

* Seventeen specimens with all tubes inoculated, contaminated.

Table 2 indicates the results in respect to the contaminations in the cultures, using different methods, and it can be readily seen that oxalic acid combined with crystal violet glycerol-potato medium gives the most satisfactory results. However, it should be kept in mind that this method does not yield the highest number of positive cultures.

Tables 3 and 4 give a comparison of the results obtained. It will be seen that nine specimens of urine were positive as judged by inoculation of guinea pigs, and only two by cultural methods. Two specimens of thoracic fluid were positive by inoculation of guinea pigs whereas none was positive by cultural methods.

Three specimens of material derived from joints, tissues, and so forth, were positive by inoculation of guinea pigs and two were positive by culture.   One culture of acid-fast bacilli, appearing at the end of forty days, appeared identical with cultures of bacilli of tuberculosis, as did one culture of material obtained from a shoulder joint which appeared positive at the end of twenty-four days.   In both instances, the comparable pigs were negative. Unfortunately these cultures were not injected into guinea pigs, for they were considered in every way typical.   Clinically, neither patient from whom the material was obtained had tuberculosis; the first clearly had adenocarcinoma of the lungs, which was

TABLE 3

Comparison of Results

(All material microscopically negative)

MATERIAL	SPECIMENS	GUINEA PIGS POSITIVE	CULTURES POSITIVE
Urine...............................	84	9	2
Chest fluid..........................	6	2	*
Joints, tissues, etc..................	10	3	2*
Total..............................	100	14	4

* One specimen of chest fluid and one specimen of fluid from a joint yielded acid-fast bacilli.   Both patients from whom these specimens were derived were nontuberculous, clinically and pathologically, and guinea pigs were negative.

proved by surgical removal of a specimen.   The second was a typical case of syringomyelia with a Charcot shoulder joint.   It becomes evident from these tables that the scarcity of bacilli of tuberculosis in the material delayed the time of the positive cultures considerably, so that from thirty to sixty days elapsed before the diagnosis was made.   In some instances the guinea pigs appeared to be positive before the cultures were positive.   Guinea pigs were permitted to live until eight weeks had elapsed in this experiment.

A certain number of failures is encountered, both in inoculation of guinea pigs and in cultures; the failures in cultures are due to over-growth of contaminants.   Enough over-growth to prevent

TABLE 4

COMPARISON OF RESULTS OBTAINED FROM MICROSCOPICALLY NEGATIVE MATERIAL

TEST	MATERIAL	DAYS BEFORE GUINEA PIG WAS POSITIVE	DAYS BEFORE CULTURE WAS POSITIVE	TUBES INOCULATED	TUBES POSITIVE ACID-FAST BACILLI	DIAGNOSIS WITH REFERENCE TO TUBERCULOSIS
1	Joint fluid (ankle)	28	30	10	1*	Tuberculosis, astragalo-calcaneal joint (surgical pathology)
2	Urine	53	30	8†	3‡	Bilateral renal tuberculosis (clinical)
3	Pus, hip	48	40	8§	1‡	Tuberculosis, hip (clinical)
4	Urine	19	60	8	1‡	Bilateral renal tuberculosis (clinical)
5	Joint fluid (wrist)	33	Neg.	8†		Tuberculosis, wrist (surgical pathology)
6	Chest fluid	25	Neg.	8†		Pulmonary tuberculosis (clinical)
7	Urine	54	Neg.	8		Left tuberculous epididymis (surgical pathology)
8	Urine	53	Neg.	8†		Bilateral renal tuberculosis (clinical)
9	Urine	36	Neg.	8†		Bilateral renal tuberculosis (clinical)
10	Urine	53	Neg.	8†		Same patient as in Test 2
11	Urine	48	Neg.	8§		Bilateral renal tuberculosis (clinical)
12	Urine	53	Neg.	8§		Same patient as in Test 2
13	Urine	31	Neg.	8†		Same patient as in Test 7
14	Chest fluid	56	Neg.	8		Pulmonary tuberculosis, pleurisy (clinical)
15	Chest fluid	Neg.	40	8†	3¶	Adenocarcinoma, lungs (surgical pathology)
16	Joint fluid (shoulder)	Neg.	24	8†	1*	Syringomyelia, Charcot (shoulder) (clinical)

* 1 tube crystal violet-glycerol-potato.
† Some tubes contaminated.
‡ 3 tubes veal-milk-cream-egg.
§ All tubes contaminated.
¶ 2 tubes veal-milk-cream-egg, 1 tube crystal violet-glycerol-potato.

diagnosis occurred in all tubes in 17 per cent of the cases, whereas no failures occurred with use of guinea pigs because at least one animal lived twenty-one days after inoculation. This, however, does not necessarily mean that when one guinea pig survived longer than twenty-one days, in the other guinea pig, which was a failure, tuberculosis might not have developed had the animal lived long enough. Of the 200 guinea pigs inoculated, thirteen

TABLE 5

Subcutaneous and Intraperitoneal Injections 1000 Guinea Pigs (500 Specimens)

(Only positive animals compared)

METHOD OF INJECTION	RESULT	NUMBER OF SPECIMENS
Subcutaneous... Intraperitoneal......................................	Positive Positive	31
Subcutaneous... Intraperitoneal......................................	Positive Negative	6
Subcutaneous... Intraperitoneal......................................	Negative Positive	9
Subcutaneous... Intraperitoneal......................................	Positive Failure	6
Subcutaneous... Intraperitoneal......................................	Failure Positive	6

died previous to three weeks. Our records, derived from the routine of two years of diagnostic work, disclosed 4 per cent of failures among cases in which both guinea pigs died previous to twenty-one days after inoculation.

Table 5 contains the results of inoculating 1,000 guinea pigs with 500 specimens of clinical material. These guinea pigs represented consecutive inoculations. The indication is that although a pair of guinea pigs may be inoculated with equal amounts of the same material, it sometimes happens that one is positive while the other is negative, even though they both lived long enough to have lesions.

We believe that as a method of choice in obscure cases in which tuberculosis is suspected inoculation of guinea pigs is still to be preferred to cultural methods. When material is abundant, both methods can be used, providing considerable caution is exercised in the interpretation of the cultures if they are positive before the lesions in the guinea pigs develop or if the cultures are positive and the animals are negative.

<div align="center">COMMENT</div>

The question as to whether guinea pigs, or cultures, or both ought to be used in the diagnosis of obscure lesions of tuberculosis is one that perhaps cannot be settled without taking into consideration the nature of the material and the nature of the institution in which the work is being done. When acid-fast bacilli are found by direct smear, it is usually unnecessary to pursue the matter any further. If it is necessary, the question of the type of tuberculosis present is usually the one involved. Although cultures might sometimes allow distinction to be made between avian and mammalian types of bacilli, the only method so far available for positive distinction of types of bacilli of tuberculosis is by inoculation of animals. If, after acid-fast bacilli have been found, the question arises as to whether they are bacilli of tuberculosis, and the clinical diagnosis is still obscure, one would be forced to inoculation of animals rather than to the use of cultures, since even if cultures were positive, animals would probably have to be inoculated. Hence the decision as to the choice of methods must rest with the results obtained with clinical material which is microscopically negative.

We have demonstrated, as have others, that a fairly considerable number of specimens will produce typical lesions in guinea pigs when the cultures are negative. A few specimens will apparently, in the hands of some workers, produce positive cultures when the guinea pigs are negative, but from our experience it would be quite unsafe to consider such acid-fast organisms as *Mycobacterium tuberculosis* unless they were inoculated into test animals or unless the clinical evidence was positively in favor of tuberculosis. It is admitted that if avian tuberculosis exists in

clinical material derived from human beings, inoculation of guinea pigs will be negative when the culture might be positive, but until the existence of avian tuberculosis in man is on a firmer basis than it is at present, the possibility mentioned must remain as a highly improbable source of error in work with guinea pigs.

The question as to the speed of diagnosis is an important one and evidence now at hand would favor the cultural method. The length of time required for bacilli of tuberculosis to appear in cultures varies considerably. Clairmont[3] reported that most of his appeared in from twenty-five to thirty days, using the modification of Hohn, but he recorded two cases in which they did not appear for about two and a half months. Meyer,[30] on the other hand using the same method, claimed it was unnecessary to wait even as long as ten to twenty days, since by making microscopic examination of smears from the surfaces of the slants, bacilli of tuberculosis could be demonstrated much earlier.

Woolsey[49] obtained many growths in less than ten days, and some as early as two days. Our experience would indicate that growth often does not appear in less than two months, and that it is somewhat correlated with the number of bacilli present in the material. More recently we have had an opportunity to use Herrold's medium and have been surprised to see on two occasions a fairly luxuriant growth of bacilli of tuberculosis, proved to be virulent by inoculation of guinea pigs after four and seven days, respectively. But these were both instances in which large numbers of bacilli of tuberculosis had been demonstrated in the material by direct smear. Although our experience with Herrold's medium is not very extensive, so far as present evidence is concerned one may expect this to yield growth of bacilli of tuberculosis in the shortest time of any medium yet proposed.

It is possible to diagnose tuberculous lesions in guinea pigs in as short a time as fourteen days after inoculation, and almost all guinea pigs that become positive at all will have lesions at the end of thirty days. If guinea pigs are as carefully observed as cultures have to be observed, and if tuberculin testing is employed by experienced persons, diagnosis can be made almost as quickly by guinea pig, and sometimes more quickly than by cultural

methods, when material is microscopically negative. If one demands that the culture be verified by inoculation of animals, the guinea pig method will of course be quicker. Recently, we have been testing guinea pigs as a routine, with 0.5 cc. of "O. T." tuberculin injected subcutaneously at the end of four weeks. So far this has resulted in no deaths of negative guinea pigs and in the death within twenty-four hours of nearly all positive animals. This method has been used extensively at Mt. Sinai Hospital, New York, with excellent results.

In large institutions, where a large proportion of the work is referred to the institution, it is essential that the methods used be as nearly final as possible. This is so important in such institutions that any method for the diagnosis of obscure suspected tuberculous lesions, short of experimentation with animals, is not justified in the light of our present knowledge. Such institutions will not be embarrassed by the added expense of handling guinea pigs, for all of these institutions have to use guinea pigs for other purposes. As a matter of fact, the work of one of us (W. H. F.) indicates that at least two cultural methods should be used on all specimens, if cultures are to be attempted, and he and other workers have brought out the importance of inoculating a large number of tubes. Thus, it becomes evident that the cultural method is not a cheap one, and although it is cheaper than the use of guinea pigs, its cost is not negligible.

If Woolsey and Petrik[50] are correct, twenty tubes must be inoculated in order to equal the dependability of inoculation of guinea pigs. It becomes evident that even to provide incubator room for a large number of specimens will become a problem, and the care in preparing medium and examining it will amount to a sizable item.

In small institutions, where animals are difficult or impossible to keep, cultural methods will find their greatest usefulness. It is perfectly evident that in cases in which clinical evidence strongly indicates a tuberculous condition, demonstration of acid-fast bacilli, either in direct smear or culture, is sufficient confirmatory evidence.

The question of using both methods will find a considerable

amount of support in the evidence presented. However, there is one serious danger, and that is when small amounts of material are available dividing it between guinea pigs and cultures may result in failure by both methods, because of too high dilution of the material.

The medicolegal aspect of the problem is not without interest and importance. At the present time it would be much easier to convince a jury that a given lesion was tuberculous if evidence could be submitted to the effect that in guinea pigs that had been given injections of material from the lesion, tuberculosis developed, than to submit the fact that acid-fast bacilli had been cultured and had not been further tested. The importance of animal controls in such legal questions has been strikingly brought out by the decision rendered by the court in the recent Luebeck disaster, in which the judge held that the lack of controlling the material by inoculation of animals constituted criminal negligence on the part of the chief health officer.

CONCLUSIONS

1. Guinea pigs kept in cages in a room free from tuberculous animals and cared for by healthy caretakers have practically no chance of becoming infected with tuberculosis.

2. Only under the worst conditions will spontaneous tuberculosis develop in guinea pigs, and rarely in less than ninety days. The type of lesion developing under these conditions is characteristic and different from those of experimentally inoculated animals.

3. Spontaneous tuberculosis in guinea pigs that are to be used for experimental purposes can be completely ruled out by exercising reasonable care.

4. Several recently devised methods of culturing *Mycobacterium tuberculosis* are excellent, and can be expected to yield a high percentage of growth in material in which the organism can be demonstrated in direct smear.

5. When bacilli of tuberculosis cannot be demonstrated in direct smear in clinical material, fewer growths will be obtained on culture than can be obtained by inoculation of guinea pigs.

6. Although most acid-fast bacilli that grow on these special mediums will without doubt be virulent bacilli of tuberculosis, the only way positively to prove virulence and identify species, is by inoculation of animals. For this reason, positive cultures are no more final than findings of acid-fast bacilli by direct smear.

7. While cultural methods are valuable and should be frequently used for demonstrating bacilli of tuberculosis, inoculation of guinea pigs remains the best method for proving the presence of virulent *Mycobacterium tuberculosis* in clinical material that is either known or not known to contain acid-fast bacilli.

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DYSPLASTIC GRANULOCYTEMIA*

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Although conditions causing marked leukopenia with con-comitant disappearance of the granular leukocytes from the cir-culation had been described by Brown,[1] Schwartz,[7] and Turk,[8] it was not until the publication of the observations of Schultz[5] many years later that the attention of the profession was focussed on this singular syndrome. Schultz recorded a fatal condition which occurred in females between the ages of thirty-eight to sixty, characterized by a necrotizing mucous membrane involve-ment of the oro-pharynx, by high fever, slight jaundice, rapid ex-haustion, and a very marked leukopenia with concomitant agran-ulocytosis. His patients had no involvement of either erythro-poietic or megakaryocytic systems which explained the absence of anemia and hemorrhagic diathesis. As the number of case re-ports in the literature rose it became evident that this interesting affection was not a narrow and sharply defined one. Though the *basic pathological processes might be the same, the disease manifestations were often found to be different.* Thus, cases were described which, though belonging to this symptom complex, instead of oral lesions presented rectal or genital necrosis. Though Schultz originally stated that these cases were rapidly fatal, reports of cures by X-ray therapy, transfusions, and recover-ies even without treatment were observed. Although the hema-tological findings in the early cases showed a complete replacement of the granulocytes by normal lymphocytes, recent publications refer to the presence of monocytes, myeloblasts and pathological lymphocytes.

Many names have been offered to cover the various differences

in the symptomatic picture or in the physical findings in this syndrome, such as agranulocytic angina (Friedemann) agranulocytic infection (Rose-Houser), stomatitis gangrenosa myelophthisica (Jagic-Spengler), and many others. Schultz[6] believed the term agranulocytosis most applicable because it actually describes the fundamental point in all cases, whereas all other terms describe incidental findings in single cases. The condition described in this paper may belong somewhere in this interesting disease complex and seems sufficiently interesting to warrant publication because it presents some new features of this interesting malady and because a careful study of our data has led us to believe that there must be a definite relationship between this picture and the agranulocytic syndrome.

CASE REPORT

M. K., aged 41, a tucker by occupation, was admitted to the medical service of Dr. A. A. Epstein on October 19, 1930, with the diagnosis of agranulocytosis. He complained of weakness, fever and rectal pain. The patient's friends had noticed his extreme pallor two months before hospitalization. About two weeks before admission he felt that he had fever and also experienced a heavy burning sensation in the region of the anus. Sitz baths, suppositories and other medications applied directly yielded no relief. The patient stayed at his work until two days before admission.

Physical examination. The patient was a well developed, well nourished adult male who though extremely pale, seemed comfortable and not very ill. Nose, mouth and throat were negative. Heart sounds were of good quality, regular, with a slight systolic murmur at the apex which was not transmitted. Lungs were negative. Abdominal examination revealed the liver one finger's breadth below the costal margin. No other viscera or masses were palpable. No rigidity nor tenderness was elicited. The extremities and reflexes were normal. The skin was very pale, soft, of normal texture and showed no hemorrhages.

Just to the right of the anal sphincter there was a black area of gangrene 15 mm. in diameter. Surrounding the lesion was an area of induration. Invasion of the mucous membrane of the anus had just begun.

Progress. During the patients thirty-two day stay in the hospital his sensorium was clear until the day before death. At no time did he develop any physical signs or symptoms other than fever, perianal gangrene, asthenia and pallor which were present on admission. His temperature which was 102.4°F., rose and stayed between 103 and 104 for a period of two weeks. During the last ten days of his life, his temperature was between 99 and 100. The

gangrenous condition gradually increased in size until it reached an area 12 cm. in diameter (fig. 1). This condition eventually destroyed the anal sphincter.

FIG. 1. GANGRENOUS ANAL LESION AS IT APPEARED SHORTLY BEFORE THE EXITUS OF THE PATIENT

Laboratory data. Daily blood examinations were made (see Chart 1). Repeated urinalyses showed nothing of importance. The Wassermann reaction was negative. Many blood cultures were sterile. Blood chemical tests

non-granular, leukocytes (fig. 2). The protoplasm of these cells varied from faintly basophilic to oxyphilic, whereas the nuclei were either homogeneous in character, or showed a somewhat granular network. The shape of the nuclei varied from round, oval to kidney and sausage shape. The oxydase reaction was negative. Other of these cells contained very fine, dust-like granules and occasionally some course azurophilic granules. Whereas the basophilic cells simulated atypical myeloblasts, the granular cells appeared to be monocytes. The condition of leukopenia and neutropenia existed for a period of eight days. Thereafter the leukocyte count gradually rose. Bone marrow puncture done on November 3rd showed an almost complete absence of polymorphonuclear elements (fig. 3). A very marked decrease in erythroblastic components was also evident. The predominating cell was a mononuclear cell similar to the monocytic cells encountered in the blood films. Oxydase test was slightly positive. On November 10th neutral red-janus green supravital preparations showed; 0.5 per cent myeloblasts, 2.75 per cent myelocytes—A, 9.25 per cent myelocytes—B, 0.75 per cent myelocytes—C, 1.5 per cent active polymorphonuclears, 76.5 per cent atypical polymorphonuclears; 7.75 per cent lymphocytes and 0.5 per cent monocytes. Neutrophils were atypical in as much as they showed fewer granules than usual and contained a markedly increased number of mitochondria. Their nuclei were also markedly irregular. Progress of the daily blood examinations showed that the monocytic cells seen during the early part of the disease were pathological myelocytes.

Chart 1 illustrates the daily variation in the total number of granulocytes, the percentage of neutrophils and leukocytes. On the date the chart began (October 20, 1930) blood examinations showed 2,000,000 erythrocytes, 40 per cent hemoglobin, 40 per cent pathological myelocytes, 2 per cent "staff" forms, 4 per cent segmented polymorphonuclears, 2 per cent eosinophiles, 43 per cent lymphocytes and 9 per cent monocytes. As already noted this picture remained essentially the same, with some fluctuations until October 28, 1930 when the number of granulocytes began to increase, the pathological myelocytes to diminish and the segmented polymorphonuclears to increase. There also began a diminution of the lymphocytes. Examinations on October 29 revealed 2,140,000 erythrocytes, 40 per cent hemoglobin, 27 per cent pathological myelocytes, 7 per cent "staff" forms, 34 per cent segmented polymorphonuclears, 22 per cent lymphocytes and 10 per cent monocytes. This tendency continued until at the time of death the patient had 1,860,000 erythrocytes, 34 per cent of hemoglobin, 2 per cent pathological myelocytes, 10 per cent "staff" forms, 90 per cent segmented polymorphonuclears, 6 per cent lymphocytes and 2 per cent monocytes. It is of interest to note that during the patient's last week of life his leukocytes rose to 40,400 and the neutrophils to 92 per cent. These granulocytes were markedly vacuolated, the nuclei very irregular, and their granules disordered and poorly staining.

Treatment. During the patient's stay in the hospital he received Sitz baths and various antiseptic dressings for his local condition. At no time did this
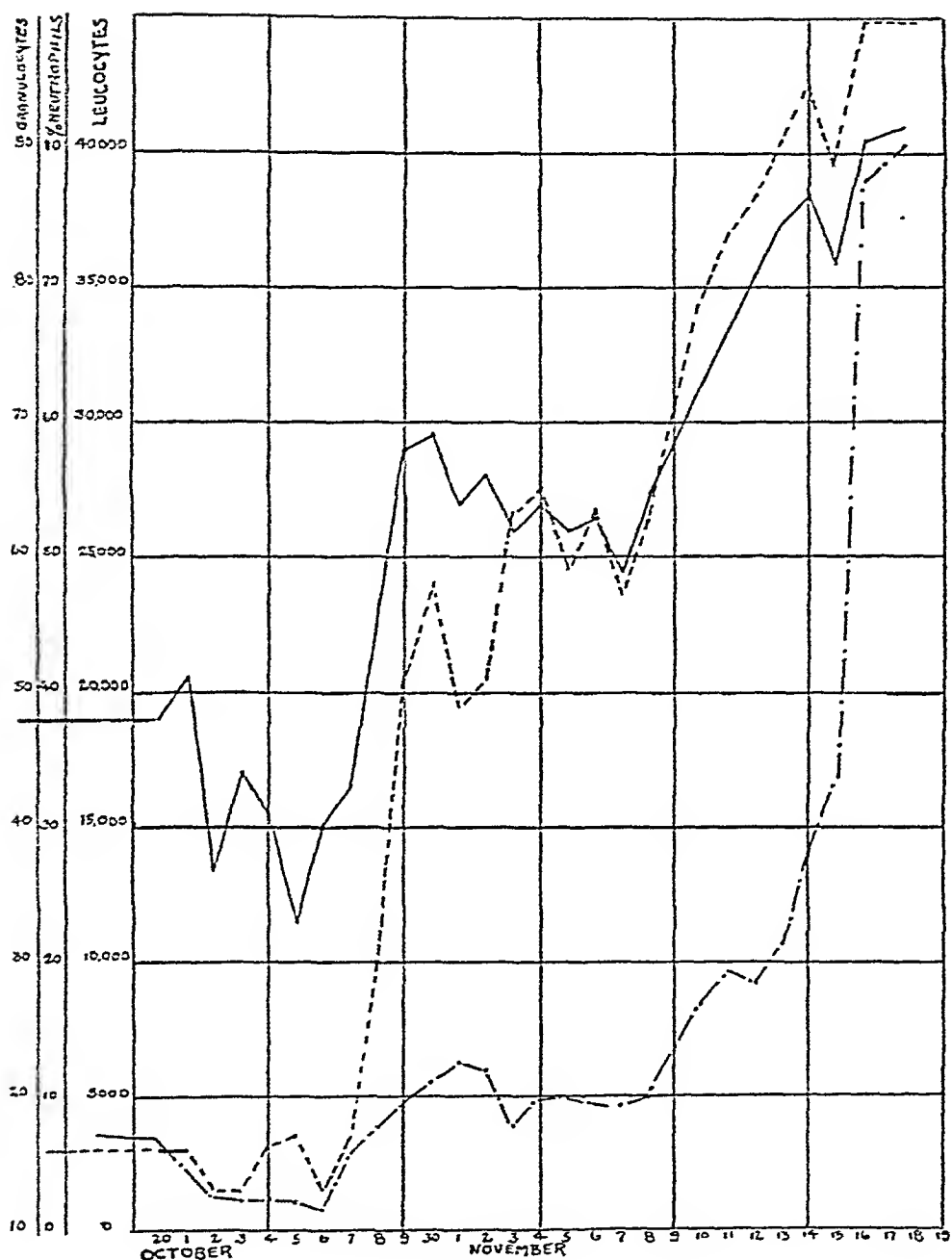
CHART 1

ually grew more asthenic, and died.   At the time of his demise his temperature
was normal and the neutrophils had returned in large numbers in the circulation.
During his illness he had shown no sign of a suppurative process, nor had he
developed a hemorrhagic diathesis.   A bone marrow puncture wound which had

healed by first intention, broke down one week before death. Smears taken therefrom showed numerous cocci in the reddish gray non-purulent discharge.

Autopsy findings. The body was that of a very pale tall strongly built man with slight edema of the lower extremities and a large black necrotic mass encircling the anus. This mass covered an area 7.5 cm. wide by 12.5 cm. long and extended deep into the tissues. There is no swelling of the lymph nodes. Heart: post mortem coagula were of a peculiar reddish color. Closing margins of the mitral valve show fine verucecae. Spleen 225 grams 16 cm. × 10 cm. × 3 cm. shows a typical wedge-shaped anemic infarct 1.3 cm. × 1.3 cm., on its lower margin. Splenic vessels were intact. There was a similar infarct at the other pole. Cut surface showed nothing unusual. The left kidney had many small hemorrhages which coalesced to form patches. Cut surface indistinct. Near the upper pole there was a mixed infarct 1 cm. deep. There was a thrombus occupying a large vein. The right kidney showed a small anemic infarct near the lower pole with a larger one near the upper, no thrombi. There were many submucous hemorrhages in the lower third of the ureter. The gasto-intestinal tract was normal except for 4 cm. of rectum near the anus which was blackish green and foul smelling. Rib bone marrow normal in porosity, extremely pale; vertebra similar, tibia (at one point) was fatty gelatinous, with very little marrow.

Microscopically the kidney showed no fatty changes, a large venous branch was thrombosed. The thrombus contained disintegrated red blood cells and many leukocytes, which were not normal. They were irregularly shaped. Surrounding the thrombus there was a wide and irregular infiltration of mononuclear elements. Many were typical plasma cells simulating a picture of acute interstitial nephritis. The glomeruli contained very little blood. The kidney showed very few hyalinized glomeruli and very little inflammatory infiltration. The splenic infarct gave the identical picture. There was nothing unusual in the cellular content of the spleen, and lymph nodes. The bone marrow of the tibia was mostly fatty, contained many indefinite mononuclear elements with dark lobulated nuclei, no erythropoiesis. A vertebra was cellular thruout, few blood vessels found, but many megakaryocytes and large mononuclear cells similar to those of the blood film were identified. The nuclei of these cells varied from round, ovoid to crescent and kidney shaped, bilobed or with many lobes. Whereas no normal neutrophils could be seen in the bone marrow, small groups are occasionally seen in the lumen of the vessels. There were few erythroblastic components.

DISCUSSION

How does the condition described compare with the agranulocytic syndrome? A review of the literature reveals as many observers who believe that agranulocytosis is primary and sepsis secondary, as those who think the sepsis is primary. Exclusive of these are the cases of paralysis of the granulopoietic apparatus

following arsenic therapy, excessive X-ray therapy and after benzol. Cases of bone marrow exhaustion have also been encountered in individuals with debility. Our patient was a strong well developed man who had had no illness. Even careful postmortem examination revealed no possible etiological cause for this condition. Except for the extensive anal necrosis the intestinal tract was normal. The findings described by Koch,[4] of a large series of post-mortem studies showed thrush-like lesions of the digestive tract with oropharyngeal necroses. It has been suggested that some agent such as faulty food may exert some selective action on the intestinal tract and secondarily on the bone marrow. The lesions in our case were in no way comparable with the findings of Koch. We feel that this case speaks for a primary affection, with an unknown causative agent.

Just what initiates the symptomatology of this condition? Bone marrow examinations in the majority of cases reported seem to indicate, in spite of their lack of uniformity, a disturbance of the bone marrow which results in a curtailment of the production of granulocytes. David[2] believes that the disturbance is not of production, but of outflow distribution. In our case we are not dealing with an agranulocytosis but with a condition which the granulocytes formed are imperfect in structure. As an inspection of the table will show, there were 33–92 per cent granulocytes present at all times, even when the leukocytes had dropped to 700 and the neutrophils to 3 per cent. The neutral red-janus green stains definitely placed the pathological mononuclears as myelocytes with deficient granules. The subsequent course of the blood count showed that these cells were the precursors of poorly granular and vacuolated neutrophils. Thus it appears that the condition resulted in the production and distribution of imperfectly constructed neutrophils which we believe were unable to assume their normal functions. For this reason we have selected the term dysplastic granulocytemia.

In the rapidly fatal cases reported in literature the granulocytes are either markedly decreased or totally absent, with a complete replacement by lymphocytes. In these cases the bone marrow is paralyzed and the granulopoietic function ceases. In

other cases, including the one under discussion, the bone marrow injury though not paralyzing, is irreparable and results in the disordered formation of granular cells. Ordinarily if the duration of the illness is protracted there is the return of what appear to be normal neutrophils. Studies of the neutrophils that appeared during the last week of our patient's life showed marked vacuolization of the protoplasm with toxic degeneration of the nucleus and granules. Whereas a marked leukopenia persisted during the early part of the illness, during the latter part, the leukocytes rose steadily. Coexisting with this severe leukocytic picture was a profound anemia. With the rise of the leukocytes there appeared a corresponding rise in the neutrophils.

Those who have reported recoveries in agranulocytosis have stressed the return of the neutrophils as the cause of recovery. Koch found the return of neutrophils with resultant recovery in 15–25 per cent of all his cases. Lack of neutrophils is considered incompatible with life. Thus X-ray therapy, transfusions, vaccines, et cetera have been used with apparent success to cause by stimulation of the bone marrow a return of the neutrophils to the circulation. Friedeman and Elkeles believe that the efficiency of X-ray therapy in cured cases can be seen by the increase in bone marrow elements during the first twenty-four hours after treatment. In our case however the return of an increased number of neutrophils did not alter the subsequent course. Every hematologist has noticed the presence of degenerated neutrophils similar to those observed in our case, somewhat before and after the peak of an acute infection. With the infectious process overcome these degenerated cells gradually disappear and the bone marrow provides normally constructed and functioning cells. During the period of stress cells as young as the myelocyte are often set out. In our case the bone marrow relieved of its pernicious influence, was able to produce neutrophils in huge quantities, but of deficient quality, because of an irreparable injury to the mother cell.

With the employment of the ordinary staining methods we believe that it is not at all unusual that these cells may be either overlooked or improperly interpreted. In this connection the

TABLE 1

Schematic Representation of the Essential Differences Found in Some of the Hematological Conditions

Disease	Erythrocytes	Leucocytes	Thrombocytes	Hemorrhagic diathesis	Prognosis
Agranulocytosis	Usually normal; below 4 million in 30 per cent of cases	Disappearance of neutrophils; often completely absent	Usually normal	Usually none; present in 17 per cent of cases	Death unless granulocytes return in normal numbers
Aleukia	Decreased	Decreased	Decreased	Present	Death
Purpura hemorrhagica	Severity of anemia depends on the amount of hemorrhage	Increased	Decreased; may be totally absent	Present	Condition controlled by splenectomy
Pernicious anemia (Addison-Biermer)	Decreased	Decreased; relative lymphocytosis	Usually normal; decreased in terminal states	Usually none; except in terminal stages	Good with adequate therapy
Secondary anemia	Decreased	Increased	Increased	None	Good with removal of etiological cause
Dysplastic granulocytemia	Decreased	Disturbed formation and function of the granulocytes. Varies from severe leucopenia to marked leucocytosis	Normal	None	Death in spite of return of granulocytes

supravital stains are of utmost importance. Table 1 summarizes the important differential points in separating several hematological conditions.

SUMMARY AND CONCLUSIONS

We have described in detail a case which demonstrates a symtom complex somewhat similar to that already described by Schultz and others as agranulocytosis. Detailed scrutiny of our case has revealed definite basic differences. The term dysplastic granulocytemia has been suggested to designate this idiopathic affliction occuring in an apparently healthy individual. It is characterized by a primary involvement of the granulopoietic system with the resultant production and distribution of imperfectly constructed neutrophils. Whereas the disappearance of the granulocytes is the central concept of agranulocytosis we encountered different findings here. Although the polymorphonuclears had dropped to 3 per cent and the leukocytes to 700, we found that during the course of the illness, granulocytes were always present amounting to between 32 and 93 per cent of the cells. Such being the case the term agranulocytosis cannot fit. Notwithstanding the fact that the leukocytes rose to 40,400 and the neutrophils to 93 per cent, and no intercurrent infection apparently intervened, the patient died. Instead of the small anal gangrenous condition improving with the return of the neutrophils, it gradually grew larger. Heretofore all of the cures in cases of agranulocytosis were attributed to the return of the granulocytes, whereas in this case a marked leukocytosis did not prevent death. The death in this case can only be explained by the inability of the neutrophils, although present in larger numbers, to assume their normal function because of an inherent deficiency in their formation.

It is because of these differences that we believe that the term dysplastic granulocytemia is more suitable for that group of cases that may fall within the description we have given. We feel that a careful morphological examination of future cases will tend to show that many of the patients presumably suffering with the agranulocytic syndrome, are in reality examples of dysplastic granulocytemia.

We wish to thank Dr. Florence R. Sabin, of The Rockefeller Institute, for her aid with the supra-vital stains, and Dr. I. W. Held for his kind suggestions.

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CLASSIFICATION OF LEUKEMIAS*

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Since routine blood counts have been introduced in hospital practice the number of recorded leukemias is rapidly increasing. It is evident that this growth is not due to a greater incidence of the disease, but to the more accurate methods of diagnosis. Leukemia can no longer be classed as a rare disease, at least by the laboratory worker.

When a diagnosis of leukemia is established, it is important to determine whether it is of the acute or chronic variety. The clinical course differs greatly in the two types. The immediate prognosis of the chronic type is generally good; the duration may be five to eight years. On the other hand, the prognosis of an acute leukemia is very grave. Seldom is the course longer than five to six months. In the majority of instances, the patient succumbs in one or two months.

The typing of leukemias into lymphoid and myeloid is of some value in the chronic leukemias. If radiation be employed as a method of treatment, the radiologist may be guided by the type of leukemia in order to concentrate on some particular tissue. This is the strongest argument in favor of the genetic typing of chronic leukemias. Aside from that, the value of the typing is mainly corroborative; if the disease is associated with splenomegaly, one would expect to find the myeloid type and if the glandular enlargement is conspicuous, the lymphoid variety is the usual finding. There are exceptions to this rule, though uncommon. The differentiation of the cell types in acute leukemia (if it could be successfully done) has no prognostic or other clinical value.

The clinician should accept the diagnosis "acute leukemia" as

a final and complete entity. Therapeutic measures would be the same regardless of the histogenetic type.

The clinical course is always the same and the logic of this subdivision even from a histogenetic viewpoint can be questioned. Any attempt to classify acute leukemias on the basis of cell types usually involves a good deal of argument, and the explanations are not always entirely acceptable.

Similar views were expressed by me[12] in a previous paper. Not possessing the authority of an academic histopathologist I could but mildly stress such views. The previous paper was based on the clinical observations and the hemotologic studies of five cases of acute leukemia. Six more cases of acute leukemia and a number of chronic cases have been studied since then. Every new case observed corroborated the above contentions, as well as do many statements in literature.

The typing of leukemias into lymphoid and myeloid is interwoven with the different theories of the origin of the blood cells. The histologist frequently seeks in leukemic cells explanations or proofs for his theories about the hematopoietic tissues. The pathologist must study the literature of the histologist and embryologist when analyzing leukemias.

A. Fränkel[4] (cited by Hirschfeld)[6] was the first to describe and formulate theories regarding acute leukemias. Naturally, he expressed only the knowledge of his times (most of his articles appeared between 1895–1898). Fränkel emphatically stated that the diagnosis of "acute leukemia" can be made from the blood picture alone. The predominance of large lymphocytes plus a leukemic high total leukocyte count was sufficient evidence to make such a diagnosis. Furthermore, Fränkel and many other contemporary hematologists considered the large lymphocyte as the mother-cell of all the leukocytes of the blood. Fränkel's views were directly opposed to Ehrlich's. The latter, a few years after Fränkel's work, laid down his well-known views. Ehrlich, Lazarus and Pinkus divided the white cells of the blood into the lymphocytes on one side and the granulocytes on the other. According to these authors lymphatic leukemia (acute or chronic) was a disease of the lymphoid tissue only. They were especially

opposed to the view that the bone marrow is also involved in lymphatic leukemia. Newman, whose views were later accepted by Walz and Pappenheim, considered the bone marrow involvement a necessary accompaniment for any leukemic blood condition. Hirschfeld[5] cited about a dozen authors, including Pappenheim, who reported cases of leukemia in which the bone marrow alone was involved. The term "mixed leukemia" was introduced. This term expressed the opinion that both myeloid and lymphoid tissues were involved. This term is often used in the German literature synonymously with "myeloid leukemia."

Summarizing his article on acute leukemias, Hirschfeld[6] expressed the opinion that taking in consideration the frequency of transition from one type of leukemia to another, one must deduct that all the leukocytes have a common origin. In an article, published a year later, he[7] discussed the histopathology of leukemia from the standpoint of both unitarian and dualistic theories. The following passages from that publication are noteworthy:

Our views about the morphologic classification of leukemias depend on the basic views about the histogenesis of leukocytes. As is known, Ehrlich, and with him many authors, accept a strictly dualistic view point. The possibility of lymphocytes being generated by the bone marrow is not admitted by them. While Ehrlich admits that there are granule-free mononuclear cells in the bone marrow, he absolutely denies that the spleen and lymph glands can generate granulocytes. He (Ehrlich), therefore, considers lymphatic leukemia as an involvement of the spleen and lymph glands only and the mixed-cell (myeloid) leukemias as an involvement of the myeloid tissue in the bone marrow. The undeniable fact that lymphocytes may be found in the bone marrow in lymphatic leukemia and that myeloid elements are found in the spleen and lymph nodes in myeloid leukemia is explained by Ehrlich on the basis of metastases that is, the blood stream will transport and colonize myeloid elements in lymphoid tissue in cases of myeloid leukemia and vice versa. On the basis of my observations [concluded Hirschfeld], I formulated different opinions which should be called unitarian.

The introduction of the myeloblast by Naegeli (a non-granular cell of the bone marrow) as the parent cell of the granulocytes was supposed to refute the claims of some contemporary hematologist (especially Pappenheim) that the "lymphoid cells" of the marrow are identical with the lymphocytes of the blood and lymphoid

tissue. Supposedly a different cell, the lymphoblast, was discovered to be the parent-cell of the lymphoid elements. This cell, morphologically very similar to the myeloblast, was claimed by Schridde to possess certain biologic characteristics distinguishing it from the latter; and so the myeloid and lymphoid elements were completely divorced. The position of the dualists seemed to be unvulnerable.

The similarity of the lymphoblast and myeloblast, however, is so great, that even in the camp of the dualists soon expressions were made about the difficulty to differentiate them. Butterfield, Heineke and Meyer,[1] (cited by Downey[2]) although accepting the dualistic views, stated that all the morphologic characteristics claimed for the lymphoblast by Naegeli and Schridde were also to be found in the myeloblast, including the Altmann-Schridde granules.

The adherents of the strict unitarian view went one step further. They claimed that the lymphoblast and myeloblast were one and the same cell. Minor biologic characteristics could be explained, they contended, by the fact that they were growing in different tissues. Maximow[3] cultured lymphoid tissue in blood plasma, to which an extract of bone marrow was added. The large lymphocytes were seen to divide by karyokinesis and to differentiate step by step into (1) leukoblasts or promyelocytes, (2) granule-poor myelocytes, (3) typical granule-rich myelocytes and (4) myelocytes with horseshoe-shaped nuclei.

Maximow considered these findings as experimental proof that myelocytes will originate from lymphoblasts in a myeloid medium. He concluded that the dualistic theory obscures and complicates our conception about haemopoiesis. The unitarian teaching has much simpler explanations. Histologically identical, lymphoblasts and myeloblasts are considered the same,—being one and the same cell.

Between the extreme unitarian theorists and the strict dualists there are many noted histologists who accept a modified view.

Granting that in the healthy adult human and other mammals there exists a morphologic dualism, authors agree that in pathologic conditions, especially in leukemias, metaplasias are very

common. Downey,[3] who can be classed as a moderate dualist, agrees with Naegeli that the myeloblast is a real stem-cell of the meyloid elements of the marrow, while the lymphocytes of the lymph nodes and the spleen are regenerated by mitosis of their own kind without the intervention of the myeloblasts. Yet he takes a strong stand against Naegeli's dogmatic dualism. He attacks Naegeli's points of differentiation between a lymphoblast and a myeloblast on morphologic, biologic and histologic grounds.

It is out of the province of this paper to argue for or against any given theory of haemopoiesis. Enough had been mentioned to justify the conclusion that in leukemias, at least, a sharp line cannot be drawn between the stem-cells of the lymphoid and myeloid tissues.

It is an established fact that in the leukemias myeloid cells may be found in lymphatic tissues and vice versa. The limitations, put by nature upon healthy adult cells to develop their own kind only, do not exist in leukemias. The hemopoietic tissues revert to their embryonal state. According to Lang,[8] myeloid transformation occurs especially freely and frequently in those organs which generated myeloid elements in the embryo, namely the spleen, lymph nodes, liver, thymus, kidney and adrenals. The metaplasia is considered to be a return to the foetal blood-forming function of these organs. Furthermore, the metaplasia is autochtonous, that is it comes from certain cells of that tissue itself, not colonized by the blood stream from some distant focus. The myeloid cells develop from certain lymphoid cells, haemocytoblasts; these under physiological conditions produce only lymphocytes in lymphatic tissue, never myelocytes.

The following statement is frequently found in literature: "Though the case reported had all the characteristics of acute lymphatic leukemia, the post-mortem findings revealed it to be acute myeloid leukemia." The revelation is generally based on the myeloid infiltration of the spleen, lymph-glands, et cetera. If one could prove that the bone marrow was the only tissue involved at the onset and the lymphoid tissue secondarily colonized by the myeloid cells through the blood stream, there would be some basis for such a stand. Such proof is so far lacking.

Acute leukemias show involvement of the bone marrow, spleen, lymph nodes, liver, kidneys and other organs, in varying degrees. The differentiation of cell types is no more distinctive in the tissues than in the blood. The cells are usually large mononuclears; the nucleus comprising about 90 per cent of the entire cell. Only a narrow border of cytoplasm is present with an excentric nucleus. From two to five granules are scattered at the periphery of the nucleus. The density of the nucleus is very uneven. In the healthy adult such a cell is altogether foreign to lymphoid tissue. Similar cells may be found in the bone marrow in greater or lesser numbers. Hence the conclusion that it is a myeloid cell and the leukemia is typed as myeloid. Why not go one step further and conclude that it is an embryonal cell, a blastic cell, which characterizes the leukemia as one of the malignant acute variety? To argue that this blastic cell belongs genetically to the lymphoid or myeloid tissue often leads to a fruitless argument. First we want to establish the criterion which identifies a cell as myeloid or lymphoid.

If the criterion for considering it myeloid is based on the assumption that it was generated by the marrow, the name is fallacious. Enough proof had been cited to show such fallacy. If the criterion for considering it myeloid is based on the morphology of the cell, it has not yet been done by any worker with a reasonable degree of certainty.

As Maximow[10] puts it, "The morphologic variations between two myeloblasts are often greater than that between a myeloblast and lymphoblast." (Cited by Downey.[2]) The biologic differences, especially those based on the oxydase reaction, are uncertain. When Winkler established this reaction as characteristic for myeloid cells only, many authors of the polyphyletic school accepted it as final for differentiation of cells of doubtful morphology. At the present the specificity of this reaction for myeloid cells is denied by many. Menten[11] (cited by Downey[3]) concluded that the reaction was an absorption phenomenon dependent on properties of intracellular surfaces, and that lymphocytes gave also a well marked reaction. Graeff[5] considered this reaction common for all kinds of cells of the animal organism. He con-

cluded that the oxydase content of the cells is more quantitative than qualitative. This fact (according to Graeff) leads some observers to hasty conclusions that this or that cell does not contain any oxydase.

Gunther Wollback[14] differentiated between endogenous and exogenous oxydase. By injecting horseradish extract into the subcutaneous tissues of a white mouse an inflammatory reaction was produced and an oxydase deposit was obtained in the different types of phagocytic cells concerned in the reaction, myeloid as well as non-myeloid (histiocytes and fibrocytes). An extract of bone marrow gave similar results, although not in the same degree. He concluded that no genetic grouping of the cells should be undertaken on the basis of the oxydase content.

From a review of the literature on acute leukemias and from his own observations on twenty-eight new cases Warren[15] drew conclusions that almost coincide with those expressed here. Noteworthy are the following passages:

There is no essential difference in the clinical picture of acute leukemia, whether the case has been diagnosed as acute myelogenous or lymphogenous leukemia. The wide divergence in diagnosis in all these cases is due to the difficulty in differentiating the immature cell-forms so that it is practically impossible to separate the cases into the usual two types.

Apparently most of the acute cases on record that have been diagnosed as lymphogenous could just as well be called myelogenous leukemia or vice versa, because there is no definite difference between them in the onset, progress, signs and symptoms, blood findings and autopsy descriptions. There are differences in degree only.

Warren, however, cannot discard the traditional conception that with the aid of special stains, in expert hands, specific characteristics of leukemic cells will be demonstrated. He considers the supravital staining method of Sabin and her coworkers as the criterion of differentiation. He refers to a case of myeloblastic leukemia reported by Sabin, Austrian, Cunningham and Doan,[13] in which this method of staining determined the type of the leukemia. This case was first considered to be an acute lymphatic leukemia, on account of the predominance of cells "apparently of the lymphocytic series" in the blood. By this special method of

staining these cells were observed to mature by amytotic cell
division and give birth to a series of cells, considered by Sabin to
be myelocytes. The cells which these expert histologists con-
sidered to be "apparently of the lymphocytic series" proved to be
myeloblasts, because oxydase-positive cells were reproduced by
them. It seems more logical to me to postpone the final judg-
ment on the primary cells until someone demonstrates a similar
case in which the supravital staining will reveal mature lympho-
cytes to be the progeny instead of the myelocytes. Then only
will we have conclusive proof that the blastic cells observed were
myeloblasts in Sabin's case and lymphoblasts in the second in-
stance. It may be of interest, that about one year later[2] the
same authors in another publication on the development of the
different forms of white cells from a common stem-cell, express
themselves as follows: "The granulocytic leucocytes, monocytes,
and lymphocytes are all formed from a single stem-cell."

The discussion heretofore was centered on the two main genetic
types of cells: lymphoid and myeloid. Now a third cell type, the
monocyte, an offspring of the reticulo-endothelial cells is dis-
tinguished. Cases of monocytic leukemia and reticulo-endothe-
lial leukemia have been reported.

It is also my belief that there is no need for singling out reticulo-
endothelial or monocytic leukemia as a separate entity. If the
majority of the cells are blastic cells, characterized by large nuclei
with a sieve-like reticulum and several nucleoli, the diagnosis of
acute leukemia is justifiable. If the cell is more or less round or
slightly oval, with a narrow border of cytoplasm to one side of
the nucleus, it may be identified by some of the polyphiletists to
be a promonocyte and by others a lymphoblast or myeloblast.
There really is no morphologic distinction. The unitarian or
monophiletists would class it as a haemocytoblast, and do away
with all the other three possibilities. Occasionally one sees a
leukemia in which the cells have the same type of blastic nucleus
but the cytoplasm is very irregular in outline and very voluminous
in proportion to the nucleus. The entire cell may reach enormous
proportions, the increase being chiefly in the cytoplasm. This
originates from the reticulo-endothelial tissue and is known as a

histiocyte or clasmatocyte. It is supposed to be the parent-cell of the monocyte. In one of my last cases there was a predominance of these cells in the blood-smear. The patient was a man in the early sixties. He was admitted to the hospital with a diagnosis of "ulcerative colitis," because of the discomfort in the abdomen and the constant bloody evacuations. The leukocyte count on admission was 16,000. The smear, as mentioned, showed chiefly these peculiar cells and some mature lymphocytes; no granulocytes were seen. I considered the blastic appearance of the nucleus sufficient evidence to warrant a diagnosis of acute leukemia, disregarding the enormous size of the cell and the fantastically shaped cytoplasm. The case proved to be a highly malignant form of acute leukemia. The man lived only a little more than two weeks. Towards the end, his total leukocyte count was 60,000. The cells resembled haemocytoblasts, such as are seen in the more common types of acute leukemia.

Many observers are inclined to classify such a case as a monocytic type. From the standpoint of descriptive exactness it is perhaps in such rare types indicated, advantagenous to mention the unusual morphology of the cell. Although from every other standpoint, one should always remember the main point, namely, if the nucleus of the cell is of the blastic type, it is an acute leukemia. If the nucleus is of the more mature variety it is a chronic case. In the latter case the differentiation of the cell type is comparatively simple, and therefore, the genetic classification is possible.

Let me emphasize the important points in the diagnosis of leukemias as follows: if the majority of the cells are of the immature blastic type, as evidenced by the appearance of the nucleus, the diagnosis of acute leukemia is justified. No other classifications are necessary, irrespective of what beliefs one has about the histogenesis of the blood cells in healthy adult tissues. If, on the other hand, the cells are relatively mature, judged mainly by the absence of the nucleoli in the nucleus, the diagnosis is chronic leukemia. In the latter instance, the cells are generally sufficiently differentiated to enable one to establish the cell type.

The heretofore accepted classification of acute leukemia, ac-

cording to cell types, has no clinical value whatsoever; and even the correctness of the cytological classification in many of the reported cases is questionable. If we desire to be more radical, and to discard altogether the traditional classifications, a more exact and descriptive classification would be: "blastic" for the acute forms and "cytic" for the chronic forms. To avoid the possibility of being involved in any academic disputes, the term haemocytoblast is the most acceptable for the cell which characterizes acute leukemia. And so we could name the disease "haemocytoblastic leukemia." The chronic leukemias would always be denoted by the -cytic ending with the exact cell type modifying the nomenclature, for example, lymphocytic, myelocytic. The endings -genous and -oid, as myelogenous or lymphoid should be avoided, as confusing, if the more descriptive classification into "blastic" and "cytic" is accepted. Occasionally one sees a case of chronic leukemia of either variety, which, after running a mild course for several years, turns into the more malignant or blastic type. The blood picture then is that of a true acute leukemia. The fact that a particular case has been diagnosed in the past as chronic leukemia should not influence the pathologist; he should be guided only by the present findings. The prognosis in such cases is the same as in any other acute leukemia.

SUMMARY AND CONCLUSIONS

(1) The acute leukemic syndrome is always characterized by the same history, clinical course, blood findings and tissue changes. There are differences in degree only.

(2) The typing of acute leukemias, into myeloid and lymphoid does not add anything from a prognostic or any other standpoint. The accuracy of this typing, even from a histogenetic viewpoint, is questionable.

(3) There is no logical reason for the division of acute leukemias into different types, unless for the sake of perpetuating traditions.

(4) When the diagnosis of "acute leukemia" is made, it should sound sufficiently expressive to the clinician, from a diagnostic,

prognostic and descriptive standpoints. If a more descriptive expression is desired, the term haemocytoblastic leukemia may be recommended, indicating that blastic cells, stem-cells, are dominating the picture.

(5) For the chronic leukemias the old genetic typing may be retained because the cells are relatively mature and more differentiated.

(6) If the term haemocytoblastic be accepted for the acute leukemias, the genetic typing of the chronic variety with -cytic ending would be proper, such as lymphocytic or myelocytic.

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DISCUSSION

DR. MAX M. STRUMIA, Philadelphia, Pa.: Doctor Rubnitz' paper has indeed a number of excellent points on which I thoroughly agree with the author.

In a paper read in the fall of 1930 before the Philadelphia County Medical Society, I expressed exactly the same view in regard to the unimportance of the differentiation between acute lymphatic leukemia and acute myelogenous leukemia. I believe that there is but one form of acute leukemia and that the differentiation between the two, rather than being useless, as Dr. Rubnitz expressed himself, is erroneous.

I cannot agree with Dr. Rubnitz, however, in his remarks about the oxidase reaction. The oxidase reaction is ordinarily done without proper controls and being a rather empirical test, controls of normal fresh blood should always be carried alongside the patient's blood. I have repeatedly found, for instance, that if one follows the course of a case of acute leukemia, cells that are oxidase positive in the early course of the disease will not show the oxidase granules later. The oxidase reaction, is therefore, not to be discarded but rather to be done more carefully and its results more critically interpreted.

Nor do I agree with the common conception that acute leukemia is simply a phase of chronic leukemia or a rapidly going form. The two diseases differ vastly; the chronic leukemia being, undoubtedly, a neoplastic disease, characterized by the enormous increase of immature but differentiated cells of either the lymphatic or myelogenous series. Acute leukemia, is essentially a very acute breaking down of the bone marrow (erythrocytes, granulocytes and platelets alike being affected) with a secondary, variable, compensatory hyperplasia of either lymphocytic or monocytic (reticulo-endothelial) systems. In addition, the abnormal cells of acute leukemia are *always* indifferentiated cells.

While I agree that the name, acute leukemia, is very undesirable, yet I am personally opposed to the introduction of new terms; it is sufficient to mention that for the primordial or indifferentiated cells found in the circulating blood of patients with acute leukemia at least ten different names have been used.

THE SEPARATION FROM BLOOD GLUCOSE OF TWO NON-GLUCOSE REDUCING SUBSTANCES*

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The quantity of sugar apparent in the blood as determined by such methods as the Folin-Wu and the Myers-Bailey, includes besides the true glucose, a non-glucose reducing substance. The numerous methods for blood sugar estimation brought out in the last fifteen years have been designed with the object not only of eliminating the reduction caused by creatinin, uric acid, et cetera, but of making a close approximation to the true glucos content of the blood. Glucose may be removed from the blood in a few seconds by years fermentation and measured by the loss in reduction.[5, 7, 18] The true glucose may also be measured directly by the new Myers-Root[11] method in which the interfering substance is precipitated by zinc, or it may be determined by the Folin-Wu reagents after precipitation of this substance with the blood proteins by the copper precipitation** of Somogyi.[17] The standard methods for blood sugar are correctly accepted as clinically accurate because this non-glucose reduction is practically constant in amount in health and disease.[19] The normal range of blood sugar is about 30 mgm. per 100 cc., the normal amount by the Folin-Wu and Myers-Bailey methods is 90 to 120 mgm. per 100 cc., by the Benedict copper method 75 to 100 mgm.

GLUCID X

The amount of true glucose in blood is the same as determined by all methods, but the non-glucose substance, estimated as glucose, varies in amount by the different methods of oxidation. Fontès and Thivolle call this non-glucose reducing substance glucid X; it is the non-glucose substance described by Best[1] as containing a pentose fraction, as not being fermentable by yeast, and as being in part precipitated by phosphotungstic acid. Glucid X does not react to the reagents of Fontès and Thivolle after fermentation, and they considered it destroyed with the glucose measuring it by the difference in reduction in the tungstate and zinc filtrates.   Measured by the Folin-Wu method as the difference in reduction before and after yeast fermentation* glucid X averages about 20 mgm. per 100 cc. in terms of glucose. However glucid X is not properly described as a non-fermentable reducing substance, as it is sometimes destroyed by the briefest yeast fermentation[15] and is always completely fermented in seventy-two hours.[14]   A preferable method of estimating glucid X is therefore by the difference between the reduction in copper and tungstate filtrates using the Folin-Wu reagents.

THE Y-REDUCTION

Comparing the non-glucose reduction by different methods Pickard, Pierce and associates found that the Benedict modification of the Lewis-Benedict reduction method (it is not fair to call it a method for blood sugar) gave regularly a much greater reduction in the blood than the Folin-Wu although the amount of the body or bodies which caused this reduction over that of the true glucose and glucid X determined by the Folin-Wu varied widely in different bloods.   Calling the excess reduction the "Y-reduction," they found it ranged in the blood of twenty-five healthy, fasting students from 14 to 68 mgm. per 100 cc. an average of 40.4 mgm. per 100 cc. as glucose.   In forty-one blood samples

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* An equal quantity of a 5 per cent suspension of Fleischmann's yeast is added to whole blood, shaken, and allowed to stand.   Although one minute is sufficient, five minutes is the usual period used.   Washing the yeast has been shown to be unnecessary.   Add distilled water to make 8 volumes, then precipitants and filter.

from patients in whom kidney damage or irritation was shown by the presence of albumin and casts in the urine the Y-reducing substances averaged 58 mgm. per 100 cc. in the four cases of severe nephritis included, they averaged 108 mgm.

The Y-reduction is not due to a fault in the picrate method of blood sugar analysis, since the Myers[10] blood sugar method was found (as is well known) to check accurately with the Folin-Wu, giving only the reduction due to glucose and to glucid X. Thus the Myers-Bailey method may be used with the Benedict modification of the Lewis-Benedict to estimate the Y-reduction. This control by the Myers test answers a question as to the cause of the excess reduction which is raised by the work of Duggan and Scott[3] who showed that with the Benedict modification the formation of picramic acid varied with both the glucose and the amount of proteins to be precipitated. The Y-reduction was also shown to have no relation to the creatinin bodies, nor to an increased sensitivity of the picric acid method to glucid X. In twenty-seven blood specimens from nephritics glucid X was normal, average 20.8 mgm. per 100 cc. The Y-reduction from these bloods showed no relation to glucid X (Y-reduction 18 to 136 mgm., average 55.5 mgm.).

The Y-reduction as a difference between two picric acid methods could be considered as more satisfactorily demonstrated as being due to the presence of a reducing substance in the blood than when left as a comparison with different precipitants and different oxidizing reagents. But it was desirable to show this reduction in other filtrates by a method as sensitive as the Benedict modification. This is possible by the Ionesco[11] method, also originally offered as a method for blood glucose. This method, like the Folin-Wu, shows no reduction even from large amounts of added creatinin, nor is it affected by yeast as used for blood sugar fermentation, either in filtrates from a yeast suspension, or from yeast added immediately after the precipitants to blood. It reduces with phenolphthalein and after acid hydrolysis the filtrate must be neutralized according to a control.*

* Reduction method of Ionesco-Matiu. In a 30 cc. Erlenmeyer flask (200 x 15 mm. tubes serve as well, and are more convenient in the water bath) put 5

In the tungstate filtrate the Ionesco method gives a reduction in excess of the Folin-Wu corresponding to that of the Benedict modification, so the substance causing the Y-reduction is present in the tungstate filtrate although it is not oxidized by the Folin-Wu reagents.[13]   The reduction in the trichloroacetic acid filtrate for which the Ionesco method was originally recommended in some instances was higher than in the meta-phosphoric and tungstic acid filtrates.   Trichloroacetic acid does not precipitate the polypeptids.   Cristol[2] estimates these from the difference between the non-protein nitrogen in this filtrate and the non-protein nitrogen in the tungstate filtrate.

SIGNIFICANCE OF THE Y-REDUCTION

Pickard, Pierce and associates found the Y-reduction almost constantly increased in nephritis over the normal of 40 mgm. per 100 cc., estimating it by subtracting the apparent glucose of the Folin-Wu method from the reduction given by the Benedict modification of the Lewis-Benedict method.   Pickard compared the Folin-Wu with the Ionesco methods in the same filtrate, and with the Benedict modification, the average Y-reduction from all bloods was 67.6 and 69.4 mgm. per 100 cc. respectively.   That the Y-reduction showed a wide divergence in some bloods is not strange when the difference in the means of estimation is considered.   This reduction is probably due to several substances which precipitation by picric or tungstic acids and the oxidation by picric acid, or by ferricyanide and permanganate, may affect even in opposite ways.   The eight blood specimens from healthy

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cc. of blood filtrate (equivalent to 0.5 cc. blood), 2 cc. normal NaOH, 1 cc. ferricyanide reagent, 10 cc. distilled water.   Place in boiling water bath twelve minutes. Cool (the mixture should be light yellow).   If colorless repeat using 2.5 cc. of filtrate.   Add 5 cc. of a 20 per cent iron free H_2SO.   The color changes to water green.   From a micro-burette add N/60 $KMnO_4$ to form a pink tint.   Each cubic centimeter of N/60 $KMnO_4$ is equivalent to 100 mgm. of glucose per cent, when using the filtrate from 0.5 cc. blood.   Use a control blank, or with 5 cc. Folin-Wu weak standard glucose (without benzoic acid) and subtract the correction.   The ferricyanide reagent is composed of potassium ferricyanide 23 grams, KOH 23 grams, distilled water to make 1000 cc.   The ferricyanide reagent and N/10 $KMnO_4$ must be kept in the ice box; the permanganate keeps about three or four weeks.

individuals examined by Pickard had an average Y-reduction of 38 mgm. The variation[12] was wide varying from 5 to 75 mgm. per 100 cc. Eleven samples from nephritics averaged 66 mgm., the five severe cases included averaged 97 mgm. for the Y-reduction. (70–136 mgm. per 100 cc.) Two syphilitics gave 58 and 65 mgm., three female patients with anemia, and a hemoglobin average of 11 grams per 100 cc. gave a Y-reduction of 80 mgm. per 100 cc. Four of the patients with nephritis had an apparent blood sugar (Folin-Wu) over 120 mgm. (125–154 mgm. per 100 cc.), a higher blood sugar has long been noted as being common in

TABLE 1

ANALYSES OF BLOOD BY DIFFERENT METHODS IN VARIOUS CONDITIONS*

TYPE OF CASE	FOLIN-WU METHOD				IONESCO METHOD				Y-REDUCTION			
	Cells	Plasma	Whole blood	Serum	Cells	Plasma	Whole blood	Serum	Cells	Plasma	Whole blood	Serum
Nephritis	122	123			333	198			211	75		
Tetany		97	160			130	250			33	90	
After yeast fermentation		23†	25†			60	70					
Nephritis			100			100	170				70	
Nephritis			136		130		160	125			24	
Normal			113	100			134	127			21	27

* All results given in terms of milligrams per 100 cc.
† Glucid X.

nephritis. The substances giving rise to the Y-reduction are thus increased in nephritis and perhaps in anemic conditions, two conditions in which cellular metabolism is retarded. Table 1 gives a comparison of various reducing substances in the blood in different conditions as determined by different methods.

COMPARISON WITH GLUTATHION

Glutathion is the chief of the sulphydryl compounds that play an important part in tissue respiration forming an oxidation-reduction system in the cells. This auto-oxidizing power of glutathion depends on the presence of catalytic metals (iron,

copper) and an additional factor (Wurmser[20]), possibly existing in combination in the cells, a reason to which Fabre[5] attributes the difficulty of dissolving it in the tissues.　Glutathion has been found in nearly all the tissues including the blood where it is confined to the cells and is said to be the chief non-glucose reducing body in whole blood.　Everett[4] with a solution of glutathion equivalent to 100 mgm. per 100 cc. in the blood, got a reduction of 21 mg. as glucose with the Folin-Wu method.　He found that zinc precipitated glutathion added to blood and also the substances forming part of the "hydrolysable sugar of the blood," and thought that the reducing substance which is conveniently named glucid X, was glutathion.　There are a number of workers besides Best who have shown that glucid X is not glutathion.　Fontès and Thivolle have shown that neither the SH nor the S-S groups affect their reagents, which oxidise glucose and glucid X and Somogyi[16] said that glutathion as it exists in the blood does not reduce alkaline copper solutions.　Glutathion is present in the cells, glucid X is equally in cells and plasma.　The substances causing the Y-reduction, is chiefly in the cells, do not react to the alkaline copper reagents, and might well comprise glutathion as the largest factor.　Glucid X is completely fermented in three days, neither glutathion nor the substances causing the Y-reduction is fermented by yeast.

The average reduction of glutathion* in solutions of 50,100 and 200 mgm. per 100 cc. by the Folin-Wu method was 20 mgm. (as glucose), added to blood 21 per cent, the actual quantity being thus five times the amount recorded in terms of glucose.　By the Ionesco method the reduction in pure solutions was 63 per cent, of the real amount as glucose; added to blood, only 49 per cent. Neither boiling nor acid hydrolysis affected either the Y-reduction of bloods nor the glutathion as regards either reducing power or the amount of nitrogen present.　A solution of glutathion of 100 mgm. per 100 cc. gave a nitrogen content of 14 mgm., and when added to blood in the same amount the added non-protein nitrogen over that of the blood was on the average 13.8 mgm.　This

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* This was received through the courtesy of Drs. A. H. Sanford and E. C. Kendall of the Mayo Clinic.

agrees with the Kendall[9] formula for glutathion, and shows that glutathion added to blood is not precipitated by tungstic acid although there is a loss in reducing power with the Ionesco reagents.   That the glutathion in the cells does not reduce the Folin-Wu copper reagent shows that the glutathion is in a different state from that chemically isolated.

Peters and Van Slyke[12] state that the undetermined nitrogen makes up about a third of the total of the blood and is largely confined to the cells.   The amount of nitrogen from uric acid and

TABLE 2

METHODS FOR THE SEPARATION OF REDUCING BODIES IN THE BLOOD

TYPES OF FILTRATE	TRUE GLUCOSE (IMMEDIATELY FERMENTABLE)	GLUCID X (SLOWLY FERMENTABLE)	Y-REDUCTION (NOT FERMENTABLE)
Copper	Folin-Wu		
Tungstic acid	Folin-Wu		
	After the destruction of glucose by yeast	Folin-Wu	
		Ionesco	
	Ionesco		
Picric acid	Myers-Bailey		
Zinc	Myers-Root		
Picrate-picric acid	Benedict modification of Lewis-Benedict		

creatinin is so small as to be of little effect, the amino acid nitrogen is small and rarely changed in amount.   Considerable changes in the non-protein nitrogen then, they say, are usually due to changes in the urea nitrogen, the undetermined nitrogen, or both.

On the assumption that the reduction of the glutathion added to blood estimated by the Ionesco method, is correct for that in the blood cells, the Y-reduction calculated as wholly glutathion should be double the figures in terms of glucose.   Normal blood has a Y-reduction average of 40 mgm. per 100 cc.   This gives 80 mgm. glutathion and 11 mgm. of nitrogen per 100 cc.   Work

in progress shows that there are bloods in which this figure is too high. The Y-reduction in the plasma would also seem to be higher than compatible with a theory that was wholly caused by a substance freed from the blood cells. Other sulphydryl compounds, as ergothionine, may be included in the Y-reduction, as well as amino acids, the amino group of which is displaced by the $KMnO_4$ used to titrate the reduction of ferricyanide. Yeast contains glutathion, but the addition of even large amounts of yeast to sheep blood did not affect the Folin-Wu reduction, the "nonfermentable" glucid X figure remaining the same. After fermentation with yeast the Ionesco method shows the reduction of both glucid X and the Y-reduction. Boiling the yeast sets free reducing bodies which affect both methods in proportion to the volume of yeast, boiling a solution of glutathion did not change the reduction by either method, nor did boiling blood filtrates affect the reduction by the Ionesco method. Separation of the Y-reduction by Clausen treatment of the filtrate reported previously is erroneous.

SUMMARY

1. There is a reducing substance in the blood, chiefly confined to the cells, which gives a reduction by the Ionesco method in the tungstate filtrate in excess of the reduction of the Folin-Wu blood sugar method of an average of 40 mgm. per 100 cc. in normal blood. It may also be estimated as a comparison between other methods (table 2). It may conveniently be called the "Y-reduction."

2. As previously reported the Y-reduction is much greater in nephritis.

3. The Y-reduction is due to substances of both physiologic and pathologic interest, of which glutathion probably forms a large part.

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EDITORIAL

The Trouble with "Yaws"!

The term "yaws" is used here to designate the tropical condition which is supposed to be different from syphilis. The term "treponematosis" is used to include both syphilis, as it occurs throughout the world when recognized early and treated adequately, and yaws, which, as I view it, is syphilis operating under mediaeval conditions of personal hygiene.

Before 1500 A.D. treponematosis masqueraded under many names: leprosy, itch, mentagra, pudendagra, sycosis, psoriasis and a host of others. Epidemiologically, it is easy to see in the Great European Epidemic of Syphilis of the fifteenth century the condition which in many parts of the tropics is now called yaws. In spite of much contrary opinion, there have been many epidemics of this condition in many countries of the temperate zones during modern times.

Until about 1800 A.D. syphilis in most Anglo-Saxon countries was known as the greatpox, because it caused large and irregular scars upon its victims. This distinguished it from the smallpox, which scarred its victims with regularly placed small scars. During the fourteenth, fifteenth and sixteenth centuries practically everyone from the "Pope of Rome on his throne to the meanest scullion in Christendom" was scarred by one of these pocks.

In smallpox this scarring is accomplished not by the virus of variola per se but by vicarious bacteria, chiefly the tissue liquefying *Micrococcus aureus.* It is probable that *M. aureus* is also responsible for the major part of the mortality in variola, which is an acute disease, whereas syphilis is chronic. Otherwise the symbiotic action of the cocci is similar in the two diseases. When an initial lesion of syphilis appears, it may be heavily infected with extraneous organisms or comparatively free from them. In

either case *Treponema pallidum* invades the blood and tissues of the body while the cocci are held back by the host's defensive mechanism. When syphilides appear some are almost sure eventually to become superinfected with cocci and unless anti-luetic treatment is instituted, these cocci will start pus formation, liquefaction of tissue and ultimate scarring in many lesions.

When the forces of liquefaction and of repair are fairly well balanced there is the histologic picture of a frambesioma, that is, of granulation tissue overlying a syphiloma. In syphilis this is noted in condylomatous, circinate and rupial types. In yaws the granulations give the strawberry appearance to the frambesioma (frambesia = strawberry) while vascular changes in the papillary layer, made necessary by the stimulus to repair, give the papillary projections upward and produce the corresponding interpapillary projections of epidermis downward. The characteristic poly-nuclear miliary abscesses are situated in these interpapillary pegs of epidermis. In yaws many of these eruptions are late frambe-sides in the congenital condition and hence will not have shown a "mother yaw."

If the juices of this lesion are sucked to the surface through this pathological filter bed a yellowish fluid reeking with microscopic life, one element of which is *Tr. pallidum*, is obtained. If a man be inoculated with this "polyvalent" antigen perhaps no lesion will appear for about three weeks or after a day or so the cocci will produce an ulcer or erosion which quickly heals and then later the chancre appears. Upon the skin this is usually not sclerotic but it may be so from the start or it may never be. If the flora of the inoculum is sufficiently virulent, the ulcerative and necrotic changes may start immediately and merge into the chancre when it appears.

After the second incubation period the secondaries come and the train of symptoms and sequelae known as syphilis. If, instead of man, the normal host of *Tr. pallidum*, some other animal is inoculated with the mixed antigen, the subsequent symptoms and course will depend entirely upon the species of animal and the method of inoculation. In any case, the symp-toms are totally unlike syphilis in man.

It is as illogical as it is unscientific to inoculate this mixed antigen and then to conclude that the result is from just one element of that antigen. This, however, is what is being done in Manila. The highly contaminated material from cases of yaws inoculated into monkeys will produce symptoms in part due to the so called *Treponema pertenue* and in part to accompanying organisms. The results, however, are here interpreted as due to *Tr. pertenue* alone. Not only this but one is asked to accept for man the results obtained in this questionable manner and upon an animal not normally suffering from treponematosis. The science of medicine is in possession of adequate knowledge of the pathogeny of *Tr. pallidum* when operating in the tissues of man away from the surface of the body. In no case does it produce suppuration and liquefaction of tissue when operating in a pure state. Why, in the case of syphilis, does one discriminate in the interpretations of results and in the case of yaws remain blind to the scientific defects of experimentation.

In smallpox the total effect is due to several viruses—to the real virus of variola, which is unknown, and to cocci and other organisms. So in syphilis the scarring lesions are attributed to the combined effect of *Tr. pallidum* working underground and the contaminant liquefiers operating on the surface. It is a truism to state that if all cases of syphilis could be treated when the roseola appears, there would not be any other eruptions. Salvarsan is specific for Treponema but not for Micrococcus.

In yaws some do not make such distinctions. The skin lesions with monkey according to Schöbl are due to *Tr. pertenue* and not to the accompanying bacteriological "menagerie." Gangosa is definitely from yaws. Based on these experiments, one is asked to change his entire conception of yaws. Knowledge has been gained concerning the laws which govern the method of tissue invasion on the part of *Tr. pertenue* and its production of immunity. Even the long established terminology of yaws is changed to conform. But the trouble with this conception is that it not only violates the laws of epidemiology, but constitutes a personally-conducted, one-man, hand-picked conception of a disease which is unrecognizable from the standpoint of earlier investi-

gators.   The skin lesions in the monkey have been played up at the expense of those visceral and osseous lesions which formed such a vital part of the clinical descriptions of those masters of the subject such as Winterbottom and Numa Rat, who painted pictures of human yaws.   The seriousness of this constitutional disease as depicted by these investigators can not be "laughed off" by the "high interpretation" of a few score protocols upon monkeys and rabbits.

I have seen florid yaws around Manila Bay all the way from Parañaque to Cavite and have treated many cases of early and late yaws in the Naval Clinic for natives at Cañacao.   I have also seen early and late yaws at many places in the West Indies, particularly in Haiti and LaGonave.   I can assure readers of the American Journal of Clinical Pathology that the one differs in no wise from the other in the human being.   The trouble with yaws as a distinct disease may be categorically stated thus; it simply does not explain the known facts in the epidemiology and pathology of treponematosis.

There is some interesting psychology shown by "yaws experts." Most of us have some certain means of differentiating yaws from syphilis, but we cannot explain this so that the "man from Missouri" can use it and yet we get irritated if anyone questions our infallibility.   One investigator has a "dead shot" bone lesion about the knee joint that is given by syphilis and not by yaws or vice versa.   Another will see in the fungating lesion of yaws "pushing up through the skin" an absolutely unique type of lesion peculiar to and distinctive of yaws.   Such a writer will not believe that an exactly similar lesion occurs in untreated syphilis.   Rupial, condylomatous and circinate lesions seen in neglected syphilis require no change in description to make them typical yaws eruptions in a filthy native of the tropics.   Still a fourth authority deplores the fact that a certain distinguished author of textbooks on tropical medicine "finds himself practically convinced of the identity of yaws and syphilis."   "If this be true," he continues, "then it is the first proved example of a disease which has a symptomatology in tropical regions not found elsewhere."   This, in spite of the publication of scores of epidemics

and hundreds of sporadic cases from the syphilised populations of every parallel of latitude from the equator to the polar circles.

The most interesting bit of psychology in "yaws work," however, is that shown by medical editors. Almost universally they will turn down manuscripts which attempt to point out the glaring inconsistencies which stalk abroad everywhere in the yaws setup. Usually the bogy they get behind is that of "controversial material." Many of these questions are not points of controversy but are good common sense and orthodox medicine. Granted, however, that they are controversial, what would Galen, the great controversialist think of such an alibi if he could return to life? What answer would be given to the statement of the fact that medicine has been one long controversy since its history began to be recorded? How many centuries behind its present exalted position among the learned professions medicine would be today had it not been for controversy, is not for me to say.

C. S. BUTLER.

NEWS AND NOTICES

THE ELEVENTH ANNUAL CONVENTION OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

The Committee on Local Arrangements and the Secretary take pleasure in announcing the following general program for the meeting to be held in New Orleans May 6 to 9, 1932. The Jung Hotel will be the headquarters for the Society and the program for the Scientific Session is:

Friday Morning, May 6

The autopsy and the pathologist —Israel Davidsohn.

Evidence to support the contention that moles are of a neuroepithelial nature —A. C. Broders and Eleanor M. Fletcher.

Histopathology of methyl alcohol poisoning.—Ernest Scott.

Less common tumors of the liver.—O. A. Brines.

Some essentials to satisfactory work in allergy.—J. H. Black.

Bronchial asthma and its treatment.—Bernard McCloskey.

Laboratory diagnosis of tuberculous meningitis.—A. G. Foord.

Friday Afternoon, May 6

The action of phenylhydrazine-hydrochloride and acetylphenylhydrazine with special reference to the use of these compounds in polycythemia.— M. Bodansky, W. L. Marr and Paul Brindley.

The agglutinin content of blood following typhoid and para-typhoid immunization.—A. G. Foord.

The blood picture in pneumonia with special reference to degenerative changes of the leukocytes.—N. Rosenthan and Charles Sutro.

Contribution to the clinical pathology of diabetes.—W. G. Exton.

Viability of tubercle bacilli. Effect of the chemical treatment used in concentration technic.—J. E. Pottenger.

Further observations on amebic infection of surgical wounds.—O. B. Hunter.

Experience with the hormone test for pregnancy.—F. E. Sondern and Jerome Silverman.

The results of two years' experiment with the Friedman test.—H. L. Reinhart.

Saturday Morning, May 7

The hematopoietic system and infection.—B. Markowitz.

Lymphomatous compression of the spinal cord.—F. H. Lamb.

Bone marrow pathology in leukopenic diseases.—R. R. Kracke.

A study of the frequency in occurrence of myeloid immaturity in pernicious anemia.—F. J. Heck.

The neutrophil in pernicious anemia.—F. J. Heck and C. H. Watkins.

Hemoglobin standards.—R. L. Haden.

Saturday Afternoon, May 7

Rose Bengal test in liver function technic and results.—W. Parker Stowe.

Liver function tests.—T. B. Magath.

A study of the so-called "O" and "H" agglutinins in typhoid and endemic typhus fevers.—H. Kemp.

Symposium

Should the precipitation test for syphilis be adopted to the exclusion of complement fixation procedures?—B. S. Levine.

The present status of the serological diagnosis of syphilis.—R. A. Kilduffe. Discussion by: B. S. Levine and R. Gilbert.

In addition to a short business session on Friday morning the regular business session will be held Monday May 9 at 9 a.m.

A special feature of the program will be a complimentary Buffet Supper at the Jung Hotel Friday May 6, at 6:30 p.m. Following this supper there will be a Round Table Discussion of two important subjects: (1) The relation of the pathologist to the individual physician and to the hospital and its staff as a unit, (2) Hospital charges for clinical laboratory service. The Annual Banquet will be held on Saturday evening May 7 at the Patio Capital where Parisian chefs serve their apprenticeship. President Corpor will deliver his address on "Prospect and Retrospect" then and other speakers, yet to be announced, will address the society.

Of particular interest to the Society will be the unusual feature of a trip to the Leprosarium which is under the active supervision of Major O. E. Denney. Dr. F. M. Johns, Chairman of the Local Arrangements Committee has supplied the following interesting information concerning the Leprosarium:

While it is difficult to trace the exact origin of endemic leprosy in Louisiana, it is likely that large families of Spanish-French origin hide a sufficient number of chronic carriers to supply a considerable annual crop of acute leprous lesions that necessitate medical assistance. The strong religious nature of the Louisianians and the biblical references to the "unclean leper" undoubtedly stimulated the founding of the State institution which is operated by a Catholic nursing Sisterhood. The gradual spread of the disease and its development in other parts of the United States caused the Federal Government several years ago to take over the institution at Carville and convert it into a Federal Hospital. Dr. Denny who has been in charge for some time has made many notable contributions to the study of leprosy and has personally prepared the largest collection of color photographs of this disease. The Leprosarium is 90 miles above New Orleans on the Mississippi River, it was originally one of the aristocratic sugar plantations of ante-bellum fame and is picturesquely situated amid moss covered Louisiana water oaks.

The trip as planned will not only be of great medical interest but will carry the members of the Society through some of the most historic country in the United States. The party will leave in touring buses promptly at 9 a.m., Sunday morning and will travel across the Mississippi River and head west into the famed Louisiana bayou country where one may see not only the most modern sugar plantations of the State but some of the early iron crucibles which the negroes used more than a hundred years ago. Several typical Louisiana towns will be passed and many famous plantation homes will be seen, including that of Chief Justice White near Napoleonville. The party will be refreshed with luncheon as guests of Dr. Denney and will return by another route past the oil refineries and the new two mile wide spillway that protects New Orleans and will return to the Hotel about 7 p.m.

Tickets costing $5.00 each will be on sale at the registration desk and will be limited to members of the Society, their relatives and friends.

The Executive Committee will meet Saturday night at the Jung Hotel.

Although there have been many methods described for using brilliant cresyl blue in staining reticulocytes, the following sub-

mitted by Dr. John C. Simpson, Norristown, Pennsylvania will be found very useful:

(1) With a wax pencil draw a ring ½ inch in diameter near one end of a clean glass slide.

(2) Place a fair sized drop of 0.3 per cent alcohol solution of brilliant cresyl blue in the ring and allow it to dry in the air. The ring prevents the stain from spreading over the slide and becoming too thin. Slides prepared in this manner may be kept for many months and good results may be obtained even after as long as eight months.

(3) Touch the stain to a drop of fresh blood on the ear or finger and allow to stand until the blood turns black or dark blue. This takes place in from five to six seconds and before the blood clots.

(4) Smear the blood along the slide in the usual manner and stain with Wright's stain. No difficulty is experienced in drawing the blood across the wax pencil ring.

By this method the reticulations stand out very clearly as clumps of dotted blue lines in the erythrocytes.

Members of the A. S. C. P. should receive a great deal of satisfaction over the verdict rendered by the Court in the recent Baker trial at Davenport, Iowa. The jury found for the defendant, the American Medical Association. Much of the evidence presented upon which the decision was reached was based on the science of pathology and many members of the Society assisted the Association in presenting its side of the case.

Notice has been received of the death of Dr. George H. Fox of Binghamton, N. Y., and Dr. W. F. Thomson of Beaumont, Texas. Both were members of the Society for some time.

BOOK REVIEWS

Man and Microbes. By Stanhope Bayne-Jones. Pp. 128, 1932, Baltimore, The Williams & Wilkins Company, $1.00.

This is a popular exposition of the information which is common to all students of microbiology. Most of the material deals with bacteriology in non-technical language, and in an instructive and interesting manner. The object for which the book was prepared has seemingly been well served, namely: to give the lay reader as much reliable information as can be obtained in a book of this size, and to give it to him in language which is readily understood. Books of this type should also find their place on the high school bookshelf, since reading of them may help the student in a decision concerning his future work.

<div align="right">Luther Thompson</div>

Neoplasms of Domesticated Animals. By William H. Feldman. Pp. 410, 1932, Philadelphia and London, W. B. Saunders Co., $6.00.

In the editorial pages of the Journal, attention has been called to the value and scope of comparative pathology. It was suggested that studies in comparative pathology would yield contrastive as well as comparative results.

In this book by Feldman, the first of its kind to be published in the English language, we have what may be termed a beginning of such a study for there is no recent volume published anywhere that gives a comprehensive treatment of the neoplasms of domesticated animals. For this reason the volume will be valuable to all students of veterinary medicine, to pathologists who are at all interested in the broader aspects of pathology and to all students of cancer.

The monograph contains not only a review of the scattered material published by others but the major portion of the text is

concerned with the author's experience which covers more than 600 cases.   The tumors in these animals are clearly described.

The main feature of the book is the large number of excellent illustrations.   Of these there are 193, all of which are original; a large share of these are photomicrographs of unusually high quality.

The scheme of classification followed is that of Mallory with some slight modification and additions.

Apparently the science of pathology as related to domestic animals has not advanced beyond the state of gross and microscopic study of specimens, for little seems to be known of the pathological changes produced on the animal body outside of the gross and histological changes in the tumor.   The science of roentgenology, so extensively applied to the study and treatment of human neoplasms, has been but little used in the case of lower animals and but small reference is therefore made to its use.

The book contains a chapter on experimentally transmissible tumors and closes with a section dealing with the preservation of pathologic material.

It is to be hoped that this excellent monograph will stimulate others to study and publish their observations in this important and significant field.

PHOSPHATES IN THE SUGAR TOLERANCE TEST

D. ROY McCULLAGH AND LOUISE VAN ALSTINE

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The literature on the relationship of phosphate to carbohydrate metabolism is now so very extensive that a survey of it would constitute a research in itself. The investigations of Hartman and Bollinger[3] and earlier, that of Harrop and Benedict,[2] and of Barenscheen,[1] are perhaps the most outstanding. It is a well established fact that phosphates are involved in carbohydrate metabolism and that the amount of phosphate in the blood changes *during the assimilation of glucose.* These changes in blood phosphate can be observed either after feeding glucose or after administration of insulin. It has been suggested that the most valuable aid in the differential diagnosis of various types of disease which involve carbohydrate metabolism is the curve of inorganic blood phosphate obtained after the administration of glucose. Hartmann and Bolliger state that "abnormalities in carbohydrate metabolism may be divided into seven groups by means of the blood phosphate curve." At present we are unable to support this contention.

This paper consists of a report of a study of 230 patients to whom we have administered 100 grams of glucose by mouth and observed the changes in blood phosphate, in the hope of determining whether or not these phosphate changes are of diagnostic value.

METHODS EMPLOYED

In this study the blood sugar estimations were made by the picric acid method[7] of Benedict, and inorganic blood phosphate was determined by the method of Kuttner and Cohen.[5] It is well known that the amount of inorganic blood phosphate changes rapidly in vitro (Kay and Byrom).[4] All analyses were made immediately after the withdrawal of the blood.

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THE AVERAGE NORMAL CURVE

In chart 1 is shown the usual change in blood phosphate and sugar after the administration of 100 grams of glucose to a normal adult. The greatest depression of the phosphate occurred at the end of two hours. In the normal, the lowest point in the phosphate curve is always after the high point in the sugar curve. The decrease in blood phosphate in a normal individual after the
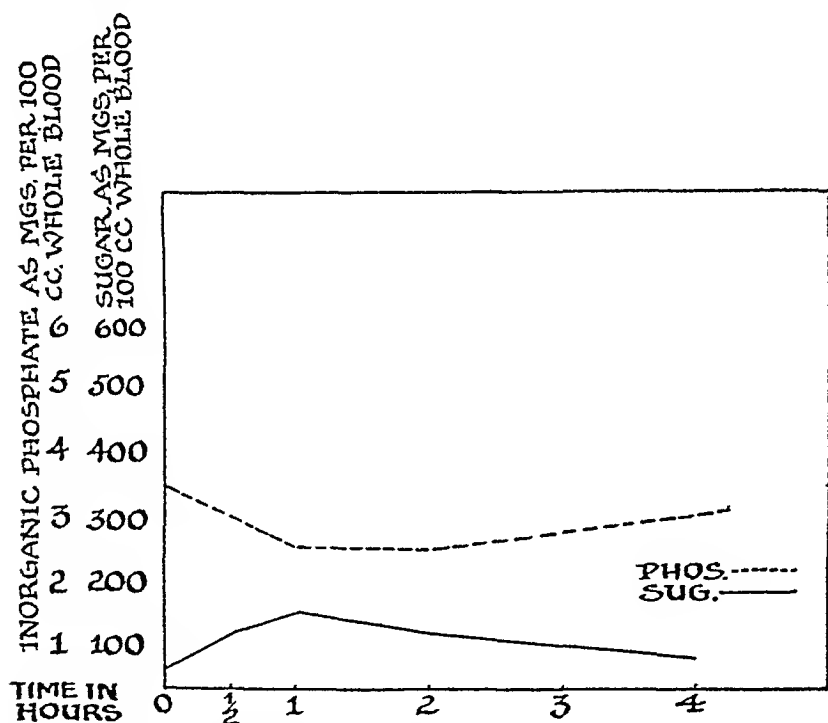

CHART 1. SUGAR AND PHOSPHATE CURVE IN AN AVERAGE NORMAL (PHOSPHATE CURVE FALLS AND RISES AGAIN)

administration of glucose averages about 0.7 mgm. per 100 cc. of whole blood, is seldom less than 0.2 mgm. per 100 cc. of whole blood, and seldom greater than 1.2 mgm. per 100 cc. of whole blood. The phosphate usually returns to nearly the original fasting level between three and four hours after the administration of the glucose.

In table 1 is shown an analysis of blood sugar and inorganic

blood phosphate curves in a series of forty-three patients in whom no known metabolic disorder was present.   In the majority of these cases the sugar curves and inorganic phosphate curves

TABLE 1

SUMMARY OF SUGAR AND PHOSPHATE CURVES IN FORTY-THREE PATIENTS HAVING No KNOWN METABOLIC DISORDER

	TOTAL	NORMAL PHOSPHATE	ABNORMAL PHOSPHATE
Low sugar curves......................	4	2	2
Normal sugar curves....................	39	32	7
Total...............................	43	34	9
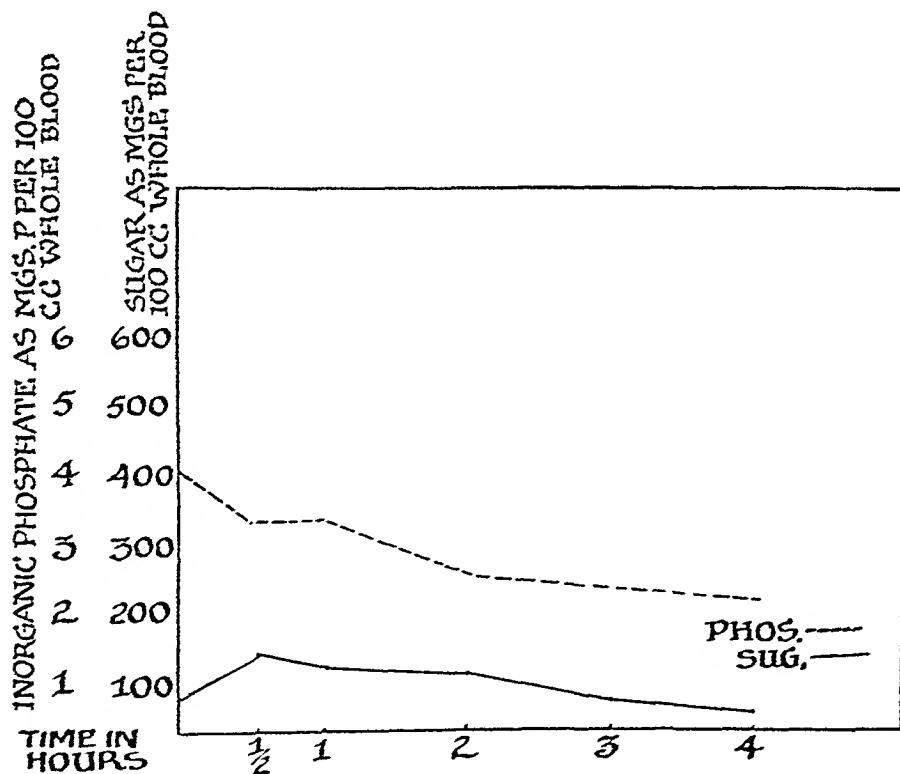
CHART 2. "DIABETIC" PHOSPHATE IN A NORMAL INDIVIDUAL (PHOSPHATE CURVE FALLS FOR FOUR HOURS)

did not deviate to any great extent from the average curve for normal individuals.   Only nine of this group of forty-three patients showed definite changes in the type of phosphate curve.

An example of such deviation from the average in a normal individual is shown in chart 2.

In a large number of diseases the phosphate curve is frequently not normal. It has been found that abnormalities of the phosphate curve are confined chiefly to diseases of metabolism, such as diabetes, hyperthyroidism, hypothyroidism, pituitary disorders, and so forth.
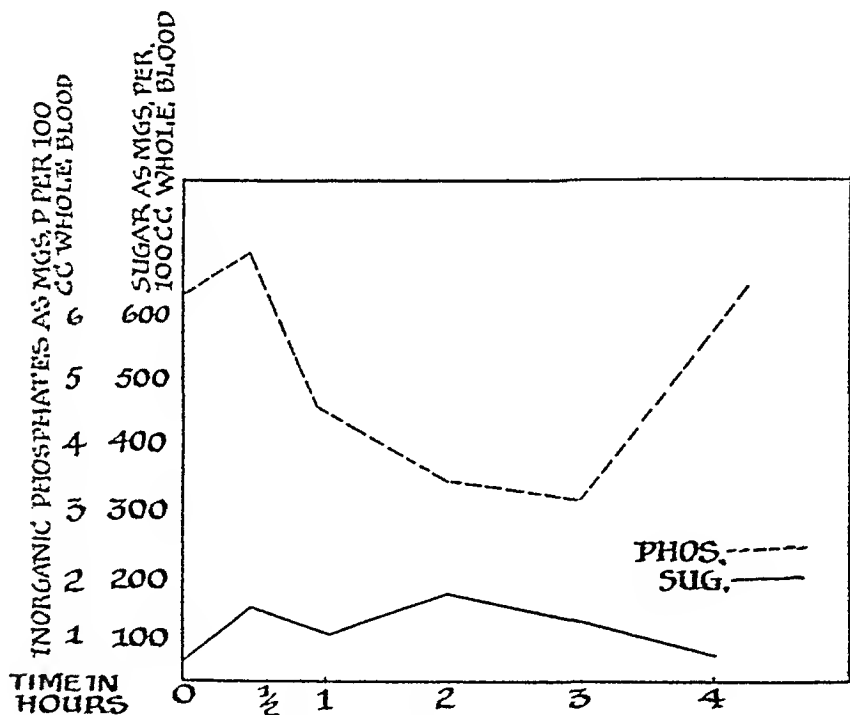
CHART 3. INORGANIC PHOSPHATE IN TETANY

TETANY

Our study of phosphate metabolism in post-operative parathyroid tetany and of the therapeutic value of the findings has been reported elsewhere.[6] In parathyroid tetany the phosphate is high and the drop is likely to be more marked than in normal individuals. When the phosphate depression is greatest, marked relief of symptoms occurs with decreased excitability as measured by electrical stimulation. The symptoms return when the phos-

phate returns to normal. This marked change is indicated in chart 3 which shows that the phosphate dropped from 6.9 to 3.4 mgm. per 100 cc. whole blood. The phosphate increased greatly again within the four-hour period. These changes are typical of this particular condition.
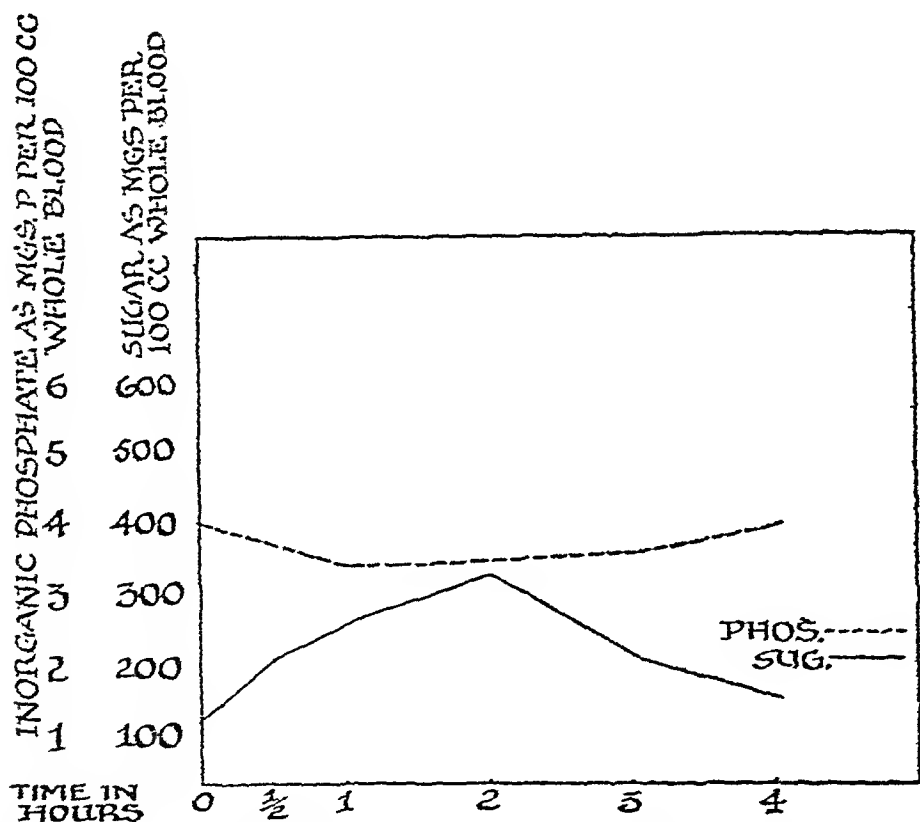
CHART 4. "NORMAL" PHOSPHATE IN A DIABETIC (PHOSPHATE CURVE FALLS AND RISES AGAIN)

DIABETES

The most marked changes in carbohydrate metabolism occur in diabetes. In a study of thirty-two patients, only 44 per cent (14 cases) or approximately one-half showed changes in phosphate curves which we could be justified in calling definitely abnormal. In 56 per cent (18 cases) the curves might well have been obtained from normal individuals. Each of these thirty-two patients was clinically definitely diabetic with a per-

sistently high sugar curve. An example of a so-called normal phosphate curve in a definitely diabetic patient is shown in chart 4. The depression of phosphate occurs at approximately the usual time and to approximately the usual extent. The return to the fasting level is also normal. The type of curve which Hartmann and Bolliger considered to be mildly diabetic is shown in chart 5. The phosphates are depressed but do not return to the fasting level before the end of the test.
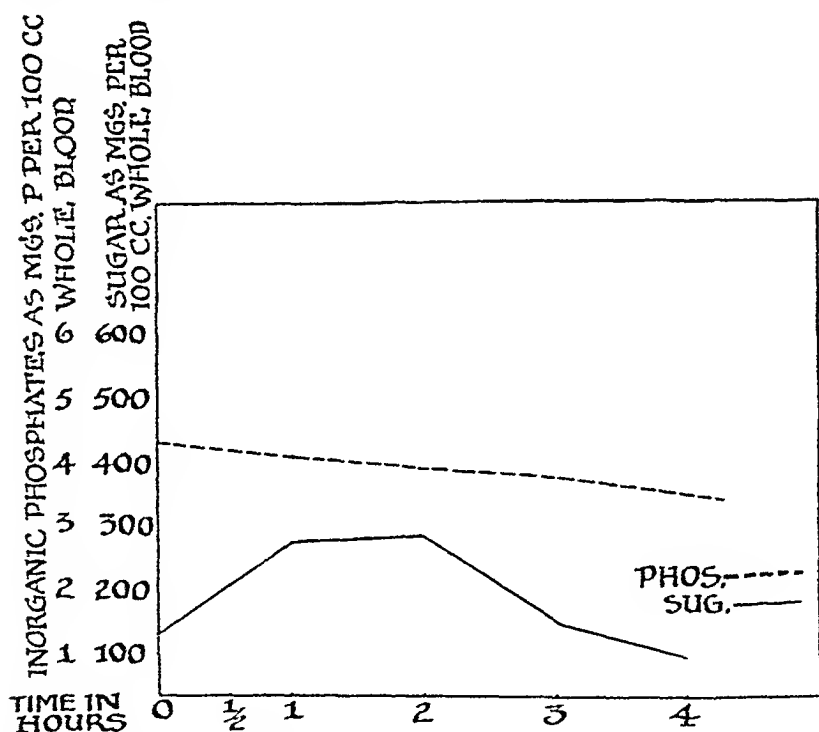

CHART 5. DIABETIC PHOSPHATE IN A DIABETIC (PHOSPHATE CONTINUES TO FALL FOR FOUR HOURS)

THYROID AND PITUITARY DISORDERS

The sugar and phosphate curves were also studied in forty-nine patients suffering from numerous metabolic disorders other than diabetes, such as hyperthyroidism, hypothyroidism, and pituitary dysfunction. In table 2 is shown an analysis of this group of curves. In thirty-two patients the sugar curves were normal, but 34 per cent of those patients showed abnormalities in phos-

phate metabolism. This percentage is definitely higher than that found in the group of patients in whom no diseases of metabolism were present. The sugar curve was high in nine cases but was not of the diabetic type. In this group of non-diabetics showing high sugar curves 33 per cent of the patients presented abnormal phosphate curves; that is a somewhat lower percentage of abnormal phosphate curves than was found in the diabetic

TABLE 2

Summary of Sugar and Phosphate Curves in Forty-nine Patients Suffering from Endocrine Disorders Other than Diabetes

	TOTAL	NORMAL PHOSPHATE	ABNORMAL PHOSPHATE
Low sugar curves:			
Hyperthyroidism......................	1	1	0
Hypothyroidism......................	3	3	0
Pituitary dysfunction.................	4	3	1
Total.............................	8	7	1
Normal sugar curves:			
Hyperthyroidism......................	8	4	4
Hypothyroidism......................	11	9	2
Pituitary dysfunction.................	13	8	5
Total.............................	32	21	11
High sugar curves:			
Hyperthyroidism......................	6	4	2
Hypothyroidism......................	1	0	1
Pituitary dysfunction.................	2	2	0
Total.............................	9	6	3
Total.............................	49	34	15

group. The shape of the abnormal phosphate curves of diabetics did not appear to be definitely different from those of the nondiabetics. Eight of the patients in the latter group presented low sugar curves, seven of whom showed normal phosphate curves.

ARTHRITIS

In forty-six cases of arthritis, table 3, sixteen patients presented definitely low sugar curves. We believe that this is not a coincidence, but that arthritics have a greater tendency toward dis-

turbed carbohydrate metabolism than most groups of patients. Seven of the sixteen arthritics with low sugar curves showed also abnormalities in phosphate metabolism. In 70 per cent of the forty-six cases the phosphate curves were normal.

TABLE 3

SUMMARY OF SUGAR AND PHOSPHATE CURVES IN FORTY-SIX CASES OF ARTHRITIS

	TOTAL	NORMAL PHOSPHATE	ABNORMAL PHOSPHATE
Low sugar	13	6	7
Normal sugar	27	22	5
High sugar	6	4	2
Total	46	32	14

TABLE 4

SUMMARY OF SUGAR AND PHOSPHATE CURVES IN PATIENTS SUFFERING FROM MORE THAN ONE DISORDER

	TOTAL	NORMAL PHOSPHATE	ABNORMAL PHOSPHATE
Arthritis and diabetes:			
High sugar curves	4	3	1
Thyroid dysfunction and arthritis:			
Low sugar curves	1	0	1
Normal sugar curves	6	5	1
High sugar curves	2	1	1
Total	9		
Thyroid dysfunction and diabetes:			
High sugar curves	13	5	8
Thyroid and pituitary dysfunctions:			
Low sugar curves	2	2	0
Normal sugar curves	3	2	1
High sugar curves	1	0	1
Total	6		

PATIENTS SUFFERING FROM SEVERAL METABOLIC DISORDERS

There were thirty-two patients in the total series of 230 who suffered from more than one disorder which we thought might affect their metabolism, namely; arthritis and diabetes, thyroid

dysfunction and arthritis, thyroid dysfunction and diabetes, and pituitary and thyroid disorders (see table 4).

In the group of patients in whom diabetes was associated with thyroid dysfunction 63 per cent of the phosphate curves were normal, which is a slightly greater percentage than that found in the diabetic patients. The shape of the phosphate curve was again not at all unique.

These patients suffering from arthritis associated with diabetes presented usual diabetic sugar curves.

In the group of patients in whom thyroid dysfunction was associated with arthritis and in the group in which both thyroid and pituitary disorders were present the number of abnormal

TABLE 5

Summary of Sugar and Phosphate Curves in Eleven Patients Affected with Miscellaneous Endocrine Dysfunctions

	TOTAL	NORMAL PHOSPHATE	ABNORMAL PHOSPHATE
Low sugar curves	4	4	0
Normal sugar curves	6	3	3
High sugar curves	1	1	0
Total	11	8	3

phosphate curves was unusually large. The shape of the phosphate curve, however, did not differentiate this group from any other group of abnormal phosphate curves.

OTHER ENDOCRINE DISORDERS

We also studied a group of eleven patients suffering from various metabolic disorders including hyperadrenalinism, ovarian dysfunction and hypometabolism. Eight patients in this series presented normal phosphate curves. The sugar curves varied as follows: four patients presented low sugar curves, six showed normal sugar curves and one a high sugar curve, which was not of the diabetic type (see table 5).

DISCUSSION

We had hoped that the phosphate curves might be of more definite value in the differential diagnosis of various metabolic

disorders. A consideration of various metabolic processes in which phosphates play some rôle leads us to believe, however, that the results of this investigation are not at all surprising. The changes in blood phosphate observed after feeding sugar are due chiefly to the part played by phosphorus in carbohydrate anabolism. Carbohydrate anabolism is very definitely disturbed in diabetes, hence we would expect frequent changes in phosphate metabolism. Phosphates, however, play a large part in the metabolism of fats, nucleoproteins, and in the metabolism of muscle. They also act as buffers in the tissue and tissue fluids. Since all of these metabolic processes are variable, it is not surprising to find considerable irregularity in the changes of the phosphates in the blood after the administration of glucose. It may be that the response would have been somewhat more regular if the glucose had been administered intravenously as in the cases studied by Hartmann and Bolliger. It seems probable, however, that in their relatively small group of patients Hartmann and Bolliger happened to get more constant results and results which are somewhat atypical. We feel that it is quite impossible to place much value on the phosphate curve in the differential diagnosis of metabolic disorders.

SUMMARY

1. The phosphate changes in the blood of normal individuals after the administration of glucose show considerable regularity.

2. The changes in blood phosphate after the administration of glucose to patients suffering from metabolic disorders frequently differ from the changes in blood phosphate in normal individuals.

3. It is impossible to make a definite differential diagnosis in various metabolic disorders by means of the phosphate curve.

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CLINICAL EVALUATION OF BLOOD PHOSPHATE AND SUGAR TOLERANCE CURVES

An Analysis of 500 Clinical Cases

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In previous publications[2,3] the experimental basis for the use of the inorganic blood phosphate curve during the sugar tolerance test was presented together with the analysis of sixty curves from different cases. This study records similar observations and the analysis of curves from 500 clinical cases of the incipient or prediabetic group. These patients had family histories of diabetes, or asthenia and poor endurance, or were overweight or underweight. Some gave a history of boils, carbuncles, paresthesias and pruritis.

With sixty curves illustrating the various types of abnormal carbohydrate metabolism already reported, it seemed that the most exacting test to which the sugar tolerance curve and the inorganic phosphate curve could be subjected would be the application to the borderline or potential diabetic group. The importance of correct diagnosis at this stage has been emphasized by Johns,[4] Sherrill[5] and others because, with early recognition of abnormal carbohydrate metabolism, severe diabetes may often be avoided through simple dietary and hygienic management. The following procedure has been applied in more than 500 instances on patients sent to the metabolic division for study.

TECHNIC

The individuals were in the post-absorbtive period and 35 grams of glucose in 50 per cent solution were injected intravenously in five to six minutes. Blood specimens were withdrawn just before the injection and fifteen, forty-five, seventy-five, and one-hundred fifty minutes after the injection. The blood sugar values were determined by the method of Folin and Wu and the blood phosphates by the method of Benedict and Theis.

In this series, more than half showed little or no abnormality in either the glucose or phosphate curves. Analysis of their histories, physical examinations and follow-ups gave no evidence of abnormal carbohydrate metabolism. Four curves from this group have been selected as typical and fifty curves have been averaged to produce a composite normal. (Fig. 1 and fig. 5, curve 1.)
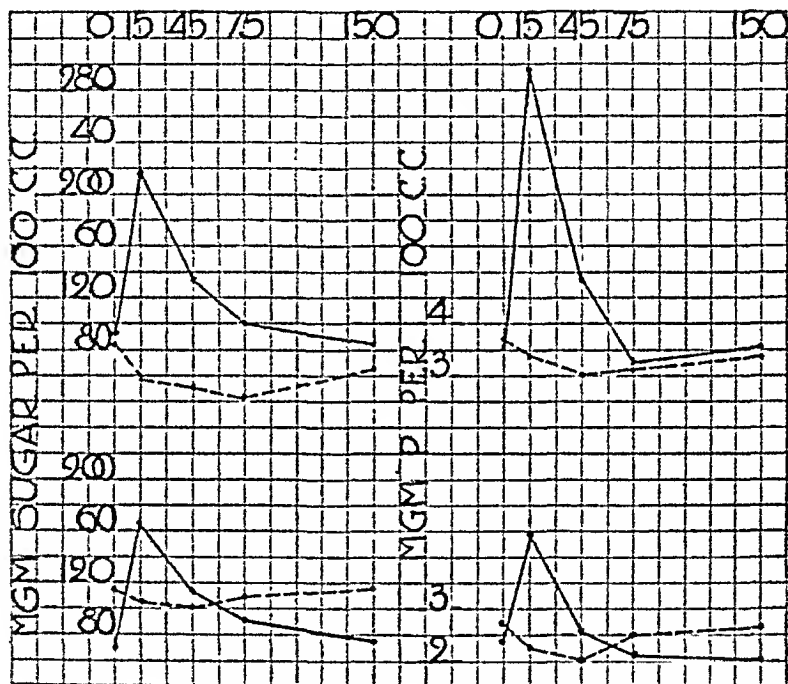

FIG. 1. FOUR GLUCOSE AND PHOSPHATE CURVES SHOWING NORMAL CARBOHYDRATE METABOLISM

In this and all other curves, time in minutes is indicated at the top of the figure.

One hundred of these patients were overweight from twenty-five to 100 pounds.' Figure 2 shows four curves which typify the group and the composite curve (Fig. 5, curve 2) representing 100 patients. If the glucose curves are contrasted with those in fig. 1 it is seen that the blood sugar rises from 20 to 100 mgm.

higher in fifteen minutes, despite the obesity in this group, and the fall is much more gradual giving a large obtuse angle. The fasting level is reached in all at the end of two and one-half hours. The curve of inorganic blood phosphates in this group shows an average drop of 0.5 mgm. with the lowest point reached
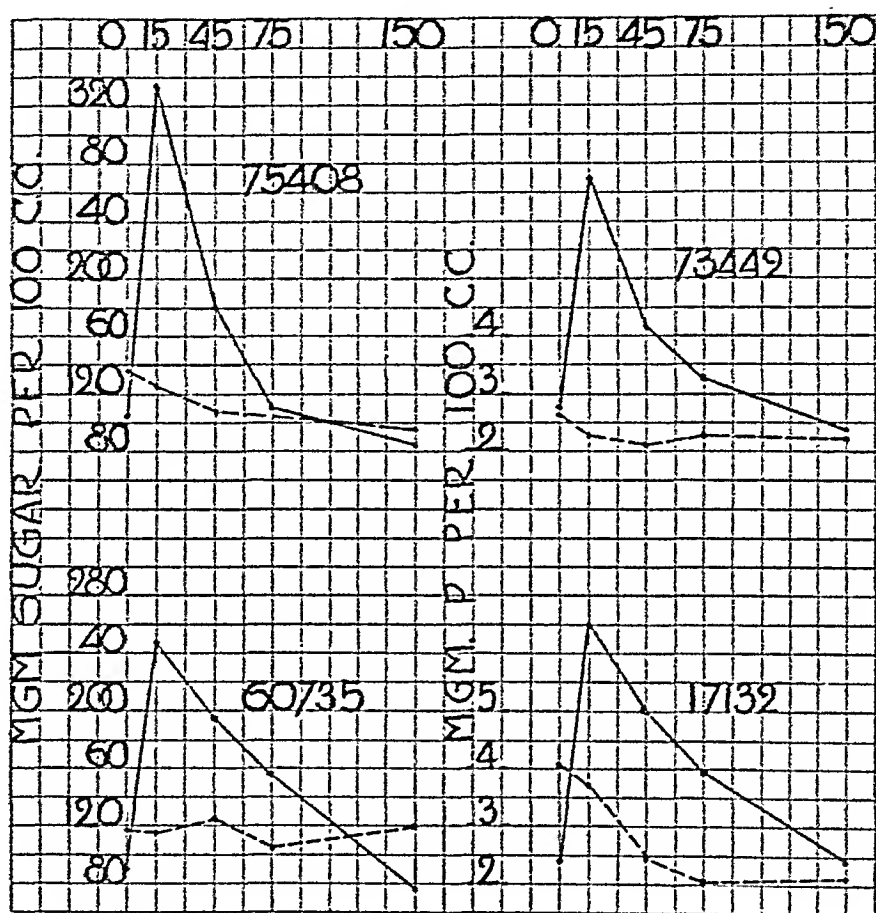
Fig. 2. Four Glucose and Phosphate Curves Showing Variations from the Normal in Obese Patients

in forty-five minutes, but there is slight tendency to recovery. In the light of experimental data the glucose curve could be explained on the basis of hepatic insufficiency but the phosphate curve indicates diminished available insulin. The clinical résumés for typical cases in this group, four of which correspond with the curves, follow:

Case 88287. Female; aged fifty-nine. Forty-three pounds overweight. Complaint: Eye trouble (cataracts). Past history: Polydipsia, polyuria, paresthesia and cramps in legs. Under dietary regulation the fasting blood sugar decreased from 119 to 94 mgm. per 100 cc. and her general condition improved.

Case 17182. Male; aged forty-eight. Twenty-nine pounds overweight. Complaint: Fatigability. Family history: Father died of diabetes. Past history: Weakness, polydipsia, in 1922 fasting blood sugar 78 mgm. per 100 cc. No glycosuria at that time. In 1928 symptoms were increased and glycosuria ++++.

Case 73442. Male; aged thirty-eight. Overweight sixty-nine pounds. Complaint: Tired feeling. Past history: Weakness and nervousness. Low caloric and low carbohydrate diet relieved symptoms.

Case 60735. Male; aged thirty-five. Overweight twenty pounds. Complaint: Recurrent conjunctivities. Past history. Fatigability, hypotension. Improvement on low caloric, low carbohydrate diet.

Case 75408. Female, aged fifty-eight. Thirty-three pounds overweight. Complaint: Pruritis vulvae. Past history. Pruritis, polyphagia, polydiasia and paresthesia. A fasting blood sugar of 118 mgm. per 100 cc. in August, 1926 was reduced to 91 mgm. per 100 cc. by March, 1928. Weight was reduced eighteen pounds by dietary regulation.

Case 94592. Female; aged sixteen. Overweight fifty pounds. Complaint: Marked weakness. Past history: Gained sixty-two pounds in ten months after menstruation began. Polyphagia, polydipsia, and dry mouth. Regulation of diet produced loss of eleven pounds in weight and brought the blood sugar down to 71 mgm. per 100 cc.

One hundred two of the patients examined were of normal weight or undernourished. Figure 3 gives curves from four average cases in this group. (see also fig. 5, curve 3). The rise of blood sugar at the end of fifteen minutes is from 88 to 238 mgm. or 43 mgm. more than the increase in the normal group (fig. 1) and 18 mgm. more than the increase in the overweight group (fig. 2). The fall of blood sugar is more gradual than the normal, leaving an obtuse angle between the two sides of the curve. This angle is more acute than in the overweight group. The phosphate curve shows only 0.25 mgm. depression, the low point is reached after one hour and fifteen minutes and there is little tendency toward recovery. Here the relatively larger amount of glucose injected apparently accounts for the greater increase in the fifteen-minute blood sugar. Probably the increased storage

accounts for the more acute fall and return to the fasting level in two and one-half hours. The phosphate curve again indicates available insulin in limited amounts. The clinical outlines for this group follow:

Case 71721. Male; aged thirty-nine. Complaint: Weakness and nervousness, polyphagia +++, polydipsia ++, polyuria ++, glycosuria ++++, asthenia +++, January, 1926 glycosuria ++++, fasting blood sugar 101
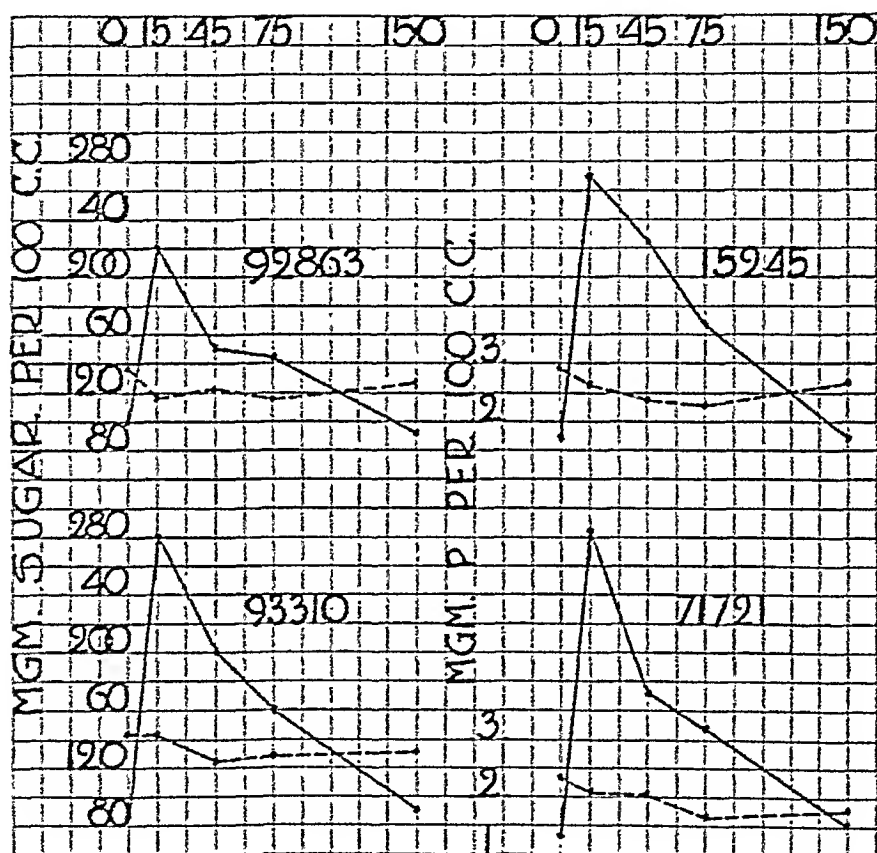

FIG. 3. FOUR GLUCOSE AND PHOSPHATE CURVES SHOWING VARIATIONS FROM THE NORMAL IN PATIENTS OF SUBNORMAL OR NORMAL WEIGHT

mgm. per 100 cc. July, 1927, after dietary regulation, fasting blood sugar 81 mgm. per 100 cc., urinary sugar negative. Felt much improved.

Case 15245. Male, aged sixty. Family history: Family all overweight, father obese, grandfather died of diabetes, sister has diabetes. Past history: Polyphagia +++, polydipsia +++, polyuria ++: Present examination: Arteriosclerosis, hypertension and marked paresthesia of extremities.

Case 93810. Male; aged thirty-two. Complaint: Weakness and vague, shifting pains. Past history: Polydipsia, polyphagia, polyuria. Twenty pounds underweight. Course: Improvement in symptoms with low carbohydrate diet. Blood sugar continued normal.

Case 47574. Female, aged forty. Complaint: Glycosuria. No symptoms suggestive of diabetes but fasting blood sugar at times reached 111 mgm. per 100 cc. Urine and blood returned to normal after dietary regulation.
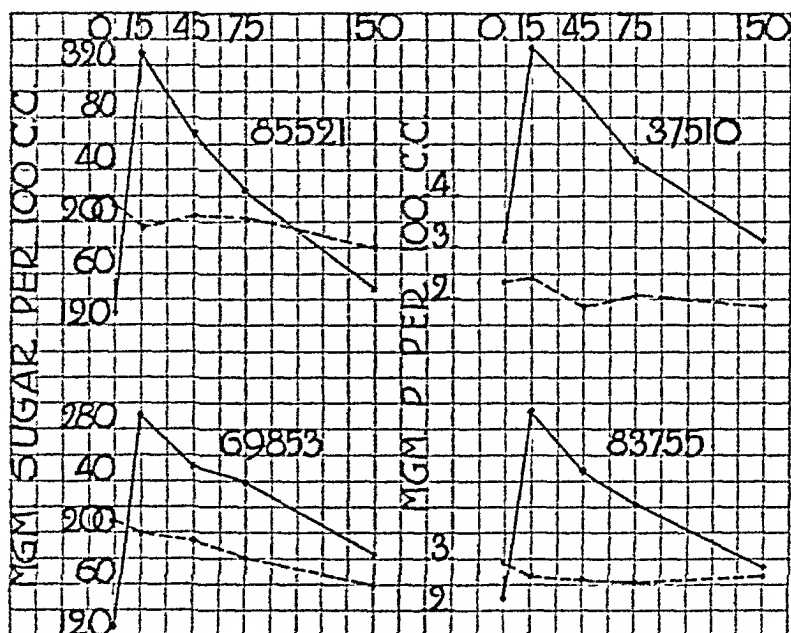

Fig. 4. Four Glucose and Phosphate Curves Showing Variations from the Normal in Patients with Diabetes

Case 54789. Male; aged fifty-five. Complaint: Impotence. Past history: Weakness and paresthesia. Present examination: Arteriosclerosis and myocarditis. Fasting blood sugar 100 mgm. per 100 cc.

Case 92803. Male; age sixty-three. Complaint: Glycosuria ten years duration. Family history: Mother obese, father died at age of 56 of diabetes. Present examination: Arteriosclerosis, hypertension, glycosuria + + +.

Although sugar tolerance tests are not indicated in diabetes, twenty-five mild diabetics were included in this study and are represented in figure 4 and figure 5, curve 4. The individual charts show the expected sharp rises in the blood sugar in the first

fifteen minutes with formation of a very obtuse angle between the two sides of the curves and tendency toward plateau formation in the descending portion of the curve. In some instances the two and one-half hour reading does not reach the fasting level. The phosphate curves show a tendency to straighten out with continued depression at the end of the observation period, indi-
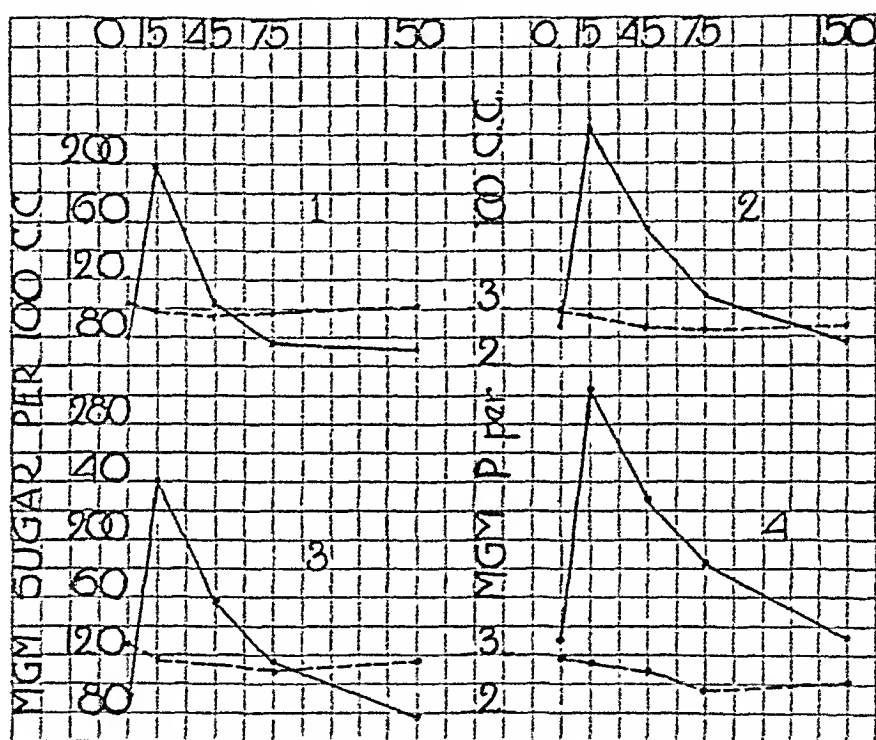

FIG. 5. COMPOSITE CURVES SHOWING GLUCOSE AND PHOSPHATE CURVES UNDER VARIOUS CONDITIONS

Curve 1, normal patients; curve 2, obese patients; curve 3, abnormal patients of subnormal or normal weight; curve 4, patients with diabetes.

cating less available insulin than in any of the groups considered. Nos. 83755 and 37510 show all the phosphate values charted on almost a straight line suggesting strongly that the diabetes was severe since similar curves were obtained on depancreatized dogs. The clinical outlines of this group follows:

Case 83755. Female; aged fifty-four. Complaint: Diabetes mellitus. Family history: No history of diabetes. Past history: Polyphagia, polydipsia,

polyuria, nocturia. Asthenia and poor endurance, dry mouth, paresthesia, pruritis. Present examination: Obese (one hundred sixty-five pounds, 20 per cent overweight). Fasting blood sugar reduced from 151 mgm. per 100 cc. to 90 mgm. per 100 cc. on diet of 2000 calories.

Case 69853. Female; aged sixty-three. Complaint: Eczema for six years. Family history: Family overweight. Father died of heart disease. Past history: Polyphagia, polydipsia. Weight 241; estimated weight 146. Boils during childhood.

Case 70434. Aged fifty-one; weight 181 pounds; estimated weight 141 pounds; blood sugar 200 mgm. per 100 cc. Complaint: Pain in stomach. Asthenia, poor endurance, occasional pruritis, "neuritis-like pains," oral sepsis, severe grade. Family history: Family all obese. No urinary sugar before tests were done. Diet: Protein, 60 grams; fat, 80 grams, carbohydrates, 100 grams, which was tolerated. Blood sugar, 83 mgm. per 100 cc., one month after treatment was started. Later dietary indiscretion caused a blood sugar of 190 mgm. per 100 cc. Weight on 5/17/27, 147 pounds; blood sugar 99 mgm. per 100 cc.

Case 87510. Age fifty-eight. Weight, 148 pounds; estimated weight, 153 pounds. Glycosuria in 1923. Classical diabetic history with all the "polys" and leg cramps, oral sepsis, chronic eczema. Diet: Protein, 50 grams; fat, 140 grams; carbohydrate, 50 grams. Blood sugar ranged between 105 mgm. and 157 mgm. per 100 cc.

Case 85521. Aged fifty-one. Complaint: "gas in abdomen." Family history: Mother obese. Past history: Hearty appetite, dry mouth numbness in hands. On restricted diet blood sugar fell from 133 mgm. per 100 cc. to 87 mgm. per 100 cc. after fasting.

Case 74516. Female, aged thirty-one. Always obese. One hundred seventy pounds at sixteen years of age; 255 pounds now; estimated weight 145 pounds. Family history: Father's weight 190 pounds; brothers' weight 190 pounds. Past history: Polydipsia at times. 3/11/26—Blood sugar after fasting 121 mgm. per 100 cc. 5/11/26–Blood sugar after fasting 99 mgm. per 100 cc. Weight now, 239 on 1000 calorie diet.

SUMMARY

(1) Five hundred combined glucose tolerance and phosphate curves were taken on patients considered potential diabetics. One hundred were from individuals twenty-five to one-hundred pounds overweight. These showed an increased rise of the blood glucose with slow fall while the inorganic phosphates decreased moderately with slow recovery.

(2) One hundred two combined glucose tolerance and phosphate curves from individuals, normal in weight or under-

nourished, showed high elevation of the glucose curve with gradual return to the fasting level while the phosphate curve showed only slight depression with slight recovery.

(3) Twenty-five combined glucose tolerance and phosphate curves on mild or moderate diabetics showed typical diminished glucose tolerance curves. The phosphate curves showed slight and continued depression.

(4) The curve of inorganic phosphates is a valuable supplement to the glucose tolerance curve in the diagnosis of abnormal carbohydrate metabolism.

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THE PATHOGENESIS OF TUBERCULOUS HEMOPTYSIS*

A CLINICAL-PATHOLOGICAL INVESTIGATION

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Hemorrhage from the lungs is the most appalling manifestation of pulmonary tuberculosis. The majority of the victims of this wide-spread disease experience this frightful symptom at least once and often repeatedly, and death results immediately in thousands of cases each year. Clinical observations of this phenomenon, consequently, have been made in abundance from the earliest times,[11] and a mere bibliographic list of the published contributions constitutes a good sized book. In spite of this wealth of recorded data and elaborate discussions, the anatomical location, the pathological processes and the physiological mechanisms that are concerned in tuberculous hemoptysis still require further elucidation.

Hemorrhage from the lungs may conceivably arise from either the bronchial or the pulmonary circulation, and may be arterial, venous, or capillary in origin. In patients who come to autopsy within a short time after a hemorrhage from tuberculosis, however, the bleeding point is uniformly located in the pulmonary circulation and practically never seen in one of the bronchial vessels, except in the rare cases of ulceration of a calcified hilum gland through the trachea or primary bronchi.[5] This is the reverse of the findings in hemorrhage from general hypertension or ulcerative bronchitis. Moreover, the bleeding vessel is found to be a branch of the pulmonary artery and not a vein or capillary, as is apt to give rise to hemorrhages in patients with mitral stenosis or pneumonia.

In the absence of necropsy observations, it cannot be proven that this also holds true in the slight streaking and initial hemor-rhages in less advanced instances of tuberculosis, plausible though this may be. Frank hemoptysis in the course of the disease, however, can be shown by roentgenogram to arise mainly from chronic fibroid phthisis with cavitation. When such patients come to autopsy, the lesions are readily demonstrated and the bleeding, when recent, is found to arise from one of the cavities.[20] This was confirmed in more than two score successive autopsies performed at the Olive View Sanatorium on patients who had had a history of hemoptysis while in the institution. Profuse tuberculous hemorrhage, particularly when fatal, always proceeds from a branch of the pulmonary artery. This also was corroborated in six fatal instances of hemoptysis at the Olive View Sanatorium.

It is often stated that the cause of pulmonary hemorrhage in tuberculosis is the erosion or ulceration of the blood vessel walls by a developing tuberculous process.[6] Since the tubercle bacillus and its products are capable of producing death with subsequent softening and liquefaction of tissues as resistant even as bone, it is assumed that the same process, acting on a blood vessel wall, may produce similar softening and consequent rupture of the vessel through which the blood can rush out. Hemorrhage, then, would be a direct result of caseation affecting the blood vessel.

If this were true, the forms of tuberculosis characterized by caseation, the exudative, rapidly progressive types in which large portions of the lungs may undergo massive coagulation necrosis in a short time, would be the most frequently affected by pulmonary hemorrhage. Experience, however, teaches that this is, in fact, the rarest of occurrences. In miliary tuberculosis, in the galloping consumption of primitive peoples coming into contact with the disease for the first time, and in the childhood type of tuberculosis in general, the absence of pulmonary hemorrhage is so striking as to be repeatedly remarked.[15] Rapid spread may, and often does, follow the hemorrhage, but hemorrhage in the course of a tuberculous process in which no resistance can be

demonstrated is indeed rare. On the contrary, it has been repeatedly noted that hemorrhage is most apt to affect the fibroid types of the disease, the proliferative forms in which the advance of the lesions are slow, and the caseation necrosis scant. In fact, it is not uncommon for apparently completely arrested cases to suffer from sudden hemorrhages.

Cloudy swelling, necrosis and liquefaction of tissues results in response to many different kinds of infection, rarely, however, is it accompanied by profuse open bleeding. The vessels in these instances are usually sealed by intravascular thrombosis and organization, with obliteration of the lumen, long before the vessel walls give way. In extrapulmonary tuberculosis the same process occurs. And more than a century ago it was pointed out that in pulmonary tuberculosis the blood vessels bordering on or traversing tuberculous cavities in the lungs are obliterated by thrombosis and endarteritis in the lumen, as well as by the compression of the contracting fibrous tissue around the adventitia. The exceptions to this observation are mainly vessels not affected by the tuberculous process, but enveloped by the resultant fibrous contraction or developed in newly forming granulation tissue.

The naïve explanation that the tuberculous process simply erodes through previously intact blood vessels, and thus produces hemorrhage is, therefore, in all probability incorrect. This applies whether the vascular injury is ascribed to ulceration and caseation due to the tubercle bacillus, its toxic products, other organisms and their toxins,[21] or the ferments and cytolytic enzymes set free from the cells already destroyed.

The idea that the blood in pulmonary hemorrhage may arise by simple diapedesis or sanguinous exudation from actively or passively dilated alveolar or bronchial capillaries as a result of vasomotor,[8] nervous or endocrine derangements in the course of clinical tuberculosis, or from toxic or mechanical factors due to the disease process itself has been advanced by a host of writers,[16] but little evidence has been submitted to support these conceptions. Changes in the composition and behavior of the blood itself have also been invoked to explain tuberculous hemoptysis and means of therapy based on these ideas have been vigorously

championed and are still widely used.[14]   Most workers have failed to confirm these claims; the clotting time and bleeding time in patients with advanced tuberculosis is more often diminished, rather than increased, and the blood clot shows normal retractility.

Pulmonary hemorrhage, when investigated on the postmortem table, is found to proceed from the wall of an old cavity in the affected lung, usually a thick, firm, fibrous wall, showing little evidence of active disease, but well organized granulation tissue with marked evidence of contracting scar and tension effects resulting from it.   Demonstrable lesions in the pulmonary blood vessels at the site of the hemorrhage have been reported by many workers during the past century.[7, 9, 12]   That most frequently noted is an aneurysm developing on a branch of the pulmonary artery lying in the wall of the cavity.[19]   Patent blood vessels in such cavity walls are often thick walled beyond the normal, and bound down by fibrous bands which may in places produce partial or complete occlusion of the lumen.   The resulting interference with the nutrition rather than any local infection of the vascular tissue itself may cause the weakening of the pulmonary arterial wall and the formation of an aneurysm.   Occasionally this aneurysm may become lined with a laminated clot undergoing organization, similar to that found in healing aortic aneurysms.   Such lesions are usually found on the larger branches of the pulmonary artery, but may develop in the smaller ones and even in the capillaries.[17]

The idea that these aneurysms develop from weakened areas on the vessel walls when the tuberculous process or other infective lesion is advancing too rapidly for the normal endarteritis and thrombosis to obliterate the lumen of the vessel, is untenable in view of the rapidity with which this latter process does occur in cases of really rapidly advancing phthisis.   The rather fantastic suggestion that the increase in fibrinogen actually present in most cases of tuberculosis forms a false coating on the inner wall of the vessel, and thus interferes with its nutrition, resulting in weakening of the vessel wall and the formation of the aneurysm is interesting but improbable.[12]   The loss of support to the

vessel wall from the excavation of pulmonary tissue which had previously occupied the cavity space is inconsiderable in view of the elasticity and ready collapsibility of the lung.

The weakening of the blood vessel wall in chronic pulmonary tuberculosis by the contracting scar interfering with the nutrition of the blood vessel itself gives only a part of the picture necessary for the production of a pulmonary hemorrhage. The other factor involved is the blood pressure existing within the vessel. It is an old observation that cerebral hemorrhage is almost confined to patients with increased intraarterial tension. It would not be surprizing, therefore, to learn that pulmonary hemorrhage occurs particularly in the presence of increased intrapulmonary arterial pressure.

Under normal conditions the pressure existing within the pulmonary vessels is low, in fact much less than half of the tension of the systemic circuit. In chronic pulmonary tuberculosis, however, much of the vascular bed through which the blood must pass in going from the right ventricle to the left auricle is closed by intravascular thrombosis and compression, as well as by actual destruction of large parts of the lung fields. Since all of the blood of the body must, nevertheless, pass through this pathway to reenter the heart, the velocity in the remaining vessels still open must be increased, and accordingly the pressure in the pulmonary artery is probably increased.

The amount of blood lost in tuberculous pulmonary hemorrhage is less than one ounce in about half of the instances reported, and rarely more than ten ounces. With repeated hemoptyses the blood loss to a patient may become considerably larger, but still is seldom sufficient to account for death from exsanguination. The immediate danger from pulmonary hemorrhage is that of suffocation from the large amount of blood coagulating and obstructing the bronchi and trachea. Instances of the removal of such blood casts of the trachea and bronchial tree have been reported, with subsequent recovery of the patient.[22]

Less dramatic but more important for the future course of the disease in most cases, however, is the aspiration of bloody material to distant parts of the lung. It has been shown that

the aspiration of sterile blood into normal lung tissues may set up considerable reaction, but this will eventually subside. When the blood is mixed with tuberculous material from the cavity contents, however, bronchiogenic spread of the disease process, sometimes pneumonic in type, is quite likely to occur.[2] Another accompaniment of pulmonary hemorrhage that is occasionally encountered consists of embolic phenomena, cerebral accidents, purpura, and so forth following the hemorrhage. This perhaps, suggests the formation of venous thrombi subsequent to infarction from thrombosis in the bleeding artery, rather than actual rupture or coagulation in a diseased pulmonary vein.

More than 4,000 instances of pulmonary hemorrhage have been recorded at the Olive View Sanatorium during the past four years, arising among 450 of the nearly 3,000 patients who have been cared for in the institution during this time. The marked periodicity in their occurrence, 80 per cent of them being reported on less than one-third of the days included in this study, suggested the possible importance of external factors in their precipitation, and the data were accordingly investigated from this point of view.[3]

The hourly variation in the incidence of pulmonary hemorrhage as revealed in this study was unexpected but unmistakable and consistent. The general impression that pulmonary hemorrhages are likely to occur during the night seems to be based more on the amount of disturbance they cause than upon their actual frequency. A confirmatory study made at the Duarte Sanatorium and analyses of other data agreed perfectly with these findings. During the walking hour, from 6:00 to 7:00 a.m., there were nearly three times as many hemorrhages recorded as during any of the hours during the night, or during the afternoon rest period.

The coughing that ensues when the patients arise from their sleep and try to expel the secretions which accumulate during the night not only involves a considerable amount of movement of the lungs and other structures, with sudden marked changes in intrapulmonary strains and stresses, but also increases the blood pressure both in the systemic and in the pulmonary circulation.

The increased activity at mealtime rather than any physiological effect of the ingestion of food may account for the increased numbers of hemoptyses seen at these hours. The lowest numbers of hemorrhages appear during the periods, both day and night, when the patients may be expected to be most completely at rest. The varying curve of vital functions during the day, the relationships of the curve of temperature, pulse and blood pressure are in accord with such a view.

The entire series showed no particular predilection for any time of the year, in marked contrast to previous observations.[1] This may be accounted for by the peculiarities of the California weather, the absence of real cold weather, freezing never having occurred at this sanatorium during the entire period, and by the consequent constancy of the environment, clothing, diet, and so forth. The variation on different days of the week is very slight; the increases on the days preceding visiting days are too small to be significant.

The incidence of pulmonary hemorrhage appears to be greatest on the days of the highest barometric pressure, and lowest on the days when the barometer is low. The uniformity of this relationship, and the absence of any significance in the mere amount of fluctuation of the barometer, either rise or fall, is in sharp contrast to the data previously reported by other workers,[4,10] but is here too marked and consistent to be disregarded. It is recalled that the systemic blood pressure increases with increase in the barometric pressure,[18] and vice versa, but that this also occurs in the pulmonary system, to any appreciable extent, although plausible, is still unproven.

Tuberculous hemoptyses also occured in greater numbers on the days of the highest maximal temperature. The relationship of the temperature to the symptoms of tuberculosis has been often discussed but is still obscure. It may be recalled that the incidence of hemoptysis need not coincide with that of the other symptoms. As noted above, few uncomfortably cold days occurred during this series.

The humidity bears no constant relationship to the incidence of pulmonary hemorrhage in this study. Since this is a dry

climate, the effects of excessive moisture may not be discerned in the figures here available. There appears to be some decrease in hemorrhages, however, with increases in the velocity of the wind, the greatest number occurring on the days with little or no air movement.

Although pulmonary hemorrhage does not necessarily indicate activity in a tuberculous patient, and may even evidence resistance and fibrosis, nevertheless its occurrence is in itself a dangerous affair, and is responsible for about one-tenth of the deaths in this group, or about 3 per cent of all the deaths in the Sanatorium by immediate suffocation or exsanguination, while a somewhat greater number died within a week or so thereafter. Thus, in the last 150 autopsies performed at the Olive View Sanatorium, in forty-three there was a record of pulmonary hemorrhage during the stay of the patient, while in fifteen there was blood in the bronchial passages or in cavities, indicating that the patients had died in hemorrhage. Every one of these forty-three cases showed fibrous or fibro-ulcerative tuberculosis with cavitation. There was not one that did not show some pleural involvement, and the pleural space was practically obliterated in nearly half of them. In the remainder there were usually dense adhesions on the side of the bleeding. Pneumothorax on the affected side, therefore, would have been impossible in practically all of the hemorrhage cases that came to autopsy. On the other hand, the hemorrhage cases that did receive successful pneumothorax treatment while at Olive View showed a somewhat lower mortality rate than those not so treated.

During the past year the bleeding point was located in six instances of fatal hemoptysis. In each case it consisted of an opening, either a linear rupture or a pinhole perforation, in a dilatation or aneurysm of a branch of the pulmonary artery. In only one instance was it in the right lung, in the remaining five it was found in the left side. In the entire series of forty-three cases, the bleeding was just twice as commonly on the left side as on the right. In one instance two small aneurysms were found on the same vessel, about a centimeter apart. In another the aneurysm arose from the pulmonary artery a short distance

from the main stem.   In no case was erosion or perforation found in a patent vein.   In each case there had been marked contraction evident in the involved lung.

A modern conception of the pathogenesis of tuberculous hemoptysis recognizes that the tuberculous process may be quiescent or even completely healed at the site of the rupture in the vessel, and that the hemorrhage is due rather to secondary changes that have occurred, both in the artery affected and in the entire circulatory-respiratory mechanism, as a result both of the disease and of climatic and other factors independent of it, instead of to the active infection caused by the acid-fast agent itself.

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A TYNDALLMETER-COLORIMETER FOR BIOLOGICAL USE AND SOME APPLICATIONS TO TURBIDIMETRIC AND COLORIMETRIC MEASUREMENTS IN MEDICINE

I. Description of the Tyndallmeter-Colorimeter for Biological Use

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It is desirable that an instrument be available for turbidimetric and colorimetric determinations on small samples of biological suspensions and solutions. It should have the following characteristics:

(1) The most sensitive optical methods of measurement should be employed in the new instrument. The instrument should be designed to utilize the great sensitivity which may be obtained by the measurement of the scattered-reflected light produced by turbid suspensions and known as the Tyndall phenomenon. (This very sensitive means of measurement has recently fallen into oblivion due to the increasing use of colorimeters as turbidimeters.)

(2) The instrument should be capable of measuring the optical properties of solutions over a wide range of dilutions.

(3) Turbidimetric and colorimetric determinations should be made by direct photometry on the substances in test tubes without removing the plug protecting the contents of the test tube and without loss of any portion of the sample, thereby obviating the present day practice of changing the substance to another vessel and immersing a plunger therein.

(4) Test tubes of varying size should be used in the same instrument for these determinations.

(5) One source of radiation (an electric lamp) should be used for both the known and unknown substances to automatically compensate for changes in the energy and other factors influencing the intensity or quality of the source of light.

(6) The standard of reference to which the turbidity or color of the suspension or solution of unknown strength should be compared should be a physical unit which will not change with time nor deteriorate.

DESCRIPTION OF THE TYNDALLMETER-COLORIMETER

The "Tyndallmeter-Colorimeter for Biological Use" was primarily designed to make possible the use of laboratory and
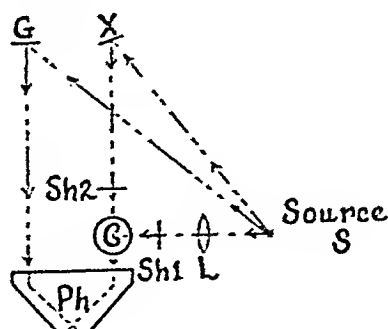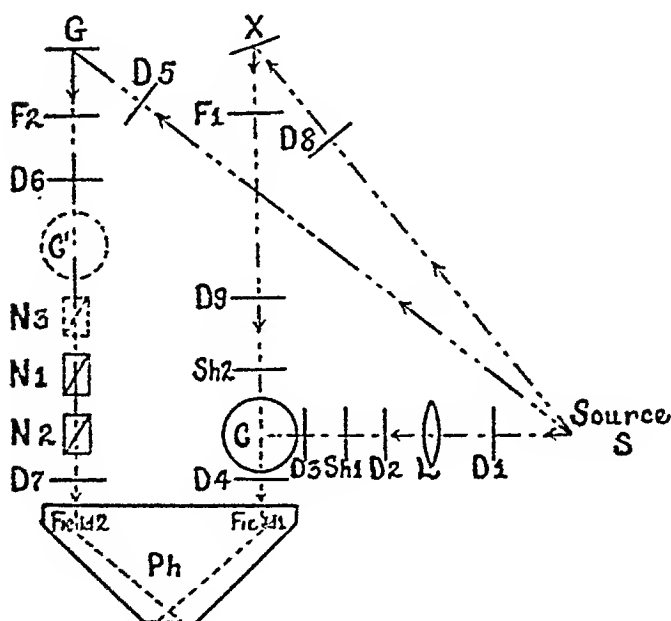

FIG. 1

FIG. 2

bacteriological test tubes as containers for the fluids to be studied and to utilize certain optical principles in the manner described. The method of photometry is optional and many methods other than that described in this paper may be used depending on the

personal preference of the operator and the requirements of the laboratory.

The essential principles of the Tyndallmeter-Colorimeter are illustrated in figure 1. The method of photometry used in this work is illustrated in figure 2. In figure 2 are also included many refinements for certain specialized problems which are not required in many routine bacteriological and clinical laboratory determinations. In the following description of the instrument, various photometric devices which may be substituted for those used in the instrument described will be suggested.

I. The optical system (figure 2)

A. As a Tyndallmeter.

The source of light (S) is a concentrated filament electric lamp and sends out three beams of light. One beam of light (hereafter to be referred to as the incident beam of light) is made parallel by means of a short focal length lens (L) and passes through a small aperture* in a diaphragm (D3) in front of cell (C). The test tube containing the turbid suspension is placed in cell (C). The scattered-reflected light produced by the turbid suspension in the test tube is observed through another small aperture* in a diaphragm (D4) in front of the side of the cell (C) adjacent to the side of the first aperture. (The cell is described in detail in section III.) This observation may be made at any angle (usually 90 degrees) with the incident beam of light.

The second beam of light coming from the source (S) falls on a suitable diffusing surface plate (G). The scattered-reflected light produced by the turbid suspension in cell (C) and the light on plate (G) are now brought into precise juxtaposition and photometered by means of the photometer cube and a system of Nicol prisms (to be described in detail in section II).

D1, D2, D3, D4, D5, D6 and D7 are diaphragms with apertures for adjusting the illuminating system. In place of these diaphragms there may be substituted diaphragmatic discs with apertures of progressive size, variable diaphragms, or absorbing glasses for increasing the measuring range of the instrument.

The axial ray of the incident beam of light formed by the source (S), lens (L), and diaphragms D1, D2, and D3 have been shown to make an angle of 90 degrees with the axial ray of Field 1 of the photometer (Ph). If it is desired to observe the scattered-reflected light produced by the turbid suspension in cell (C) at any angle other than 90 degrees with the incident beam of light, the opti-

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* These apertures may be cut into cell (C) if the Tyndallmeter is to be adjusted to always observe the scattered-reflected light produced by the turbid suspension at a fixed angle with the incident beam of light.

cal system may be mounted so as to make the desired angle; or the optical system may be mounted on an arm which is rotatable about a point where the axial ray of the optical system producing the incident beam of light intersects the axial ray of Field 1 of the photometer as a center.

This work was originally undertaken for the design of a new instrument for the study of turbidity. It was soon found that it might easily be transformed into a colorimeter. It was concluded that the combined instrument would be more desirable since it would allow its use to be extended to a larger number of clinical laboratory determinations.

B. As a Colorimeter. The tyndallmeter may be transformed into a colorimeter by closing shutter 1(Sh1), thereby preventing the incident beam of light from entering cell (C), and opening shutter 2 (SH2) which permits another beam of light coming from the source (S) and falling upon the reflecting or diffusing surface plate (X) to enter cell (C). The path of the beam of light now entering cell (C) must be at a right angle with the incident beam of light previously described. Plate (X) is rotatable so that either diffuse or reflected light may be used to determine the absorption of the fluid placed in cell (C). D8 and D9 are diaphragms similar to those described for the tyndallmeter.

The same photometers used with the tyndallmeter may be used with the colorimeter. Colorimetry may be carried out by either of the two following methods.

1. A preparation of known strength of the solution to be studied is placed in cell (C′)† having two apertures and permitting the beam of light to pass through the solution. The solution of unknown strength is placed in cell (C). In each case the solution is contained in a test tube and the test tube is placed in the cell. The intensities of the two beams of light are now measured in the manner to be described in the next section.

2. Light filters (F1) and (F2) transmitting light in the region of one of the absorption bands of the solution to be studied are placed in the path of both beams of light as indicated in figure 2. The determination of the strength of the solution of unknown strength is now made by the measurement of the

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† When the instrument is used for turbidimetry, the cell (C′) is not used except to permit a beam of light to pass through the two apertures, and the light filters (F1) and F2) are not in use.

"monochromatic" light absorbed by the solution in the region of the absorption band. The solution of known strength is no longer necessary for this measurement except for the calibration of the instrument. The properties of solutions used in clinical laboratory determinations are now being studied and suitable filters are being prepared.

II. *The photometer cube and the photometer (figure 2)*

The scattered-reflected light produced by the turbid suspension or the light absorbed by the solution in the test tube in cell (C) and the comparative source of illumination on plate (G) are brought into precise juxtaposition as two adjacent fields by means of the Duboscq‡ prism with the biprism occular (Ph), serving as a photometer cube. The brightness of the two fields is now adjusted to equality by means of the Nicol prisms (N1) and (N2). The Nicol (N1) is fixed and Nicol (N2) is rotatable. The scale is calibrated in quarter degrees of arc. The variation of the intensity (I) of field 2 is calculated from the formula $I = k \cos^2\phi$ where ϕ is the angle of rotation of Nicol (N2) measured in degrees of arc and k is a constant.

This arrangement is satisfactory in all colorimetric work. However, in turbidimetric work it is only satisfactory if the measurement of the scattered-reflected light is always made at any fixed angle with the incident beam of light. If this angle be varied and changes take place in the polarization of the light emitted from plate (G), the following precautions must be taken:

Plate (G) may be made of a substance which is a nearly perfect diffuser of light (a surface coated with magnesium oxide or magnesium carbonate, plaster of paris, or certain qualities of milk glass). Such a surface is necessary since perfectly diffuse light is unpolarized.

Another arrangement of the Nicol prisms offering a variety of measurement which is at times desirable may be mentioned. Nicol prisms (N2) and (N3) are adjusted so that their planes of maximum polarization are parallel to each other. If there is

‡ For this purpose the Lummer-Brodhun is the most desirable photometer cube, but it is more expensive. Any of the various forms of the Ritchie wedge described in Walsh[3], a Martens biprism[3], or a Bunsen[3] photometer are less expensive substitutes which may be used.

any polarized light emitted from plate (G), the common plane
of the two Nicols must be adjusted parallel to the plane of sym-
metry of the polarized light emitted from plate (G). The varia-
tion of the intensity (I) in field 2 is now calculated from the for-
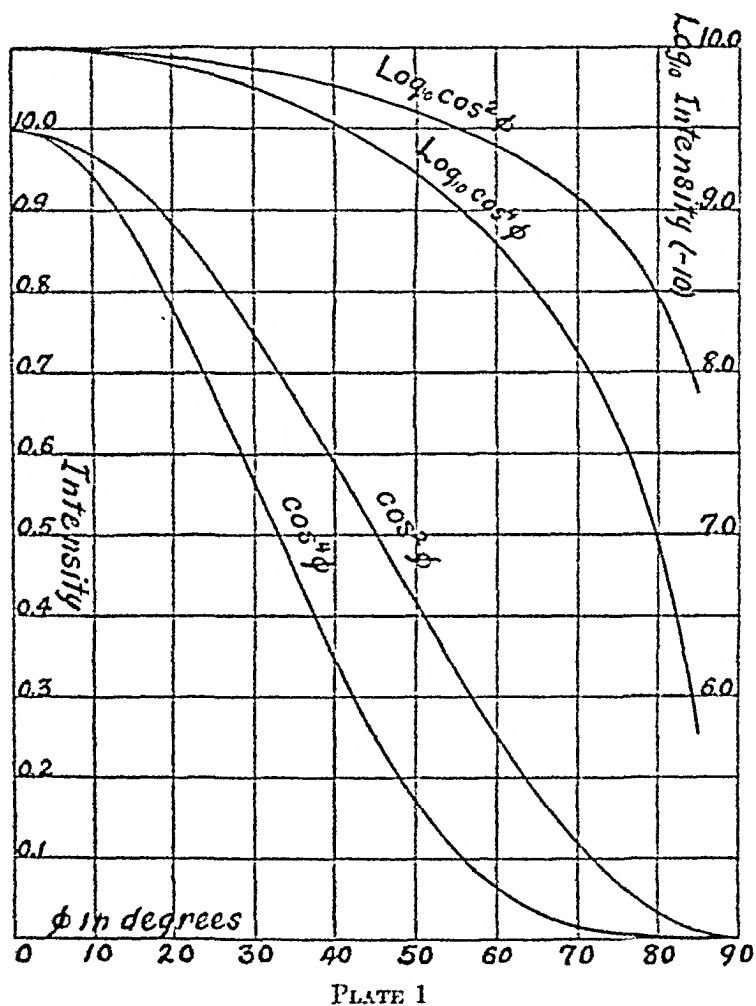
PLATE 1

mula $I = k \cos^4\phi$, where ϕ is the angle of rotation of Nicol (N1)
measured in degrees of arc and k is a constant.

The relationships between the intensities expressed in terms of
functions of $\cos^2\phi$, $\cos^4\phi$, $\mathrm{Log}_{10} \cos^2\phi$, and $\mathrm{Log}_{10} \cos^4\phi$ have been
plotted in plate 1. The curves are of value in determining the

range of measurement with any one system of diaphragms and the precision with which measurements may be made in different regions of the scale around which the Nicol is rotated. The minimum precision in any region of the scale when the intensity is a function of $\cos^2\phi$ is better than 0.5 per cent. When the intensity is a function of $\cos^4\phi$, the minimum precision in any region of the scale is 0.7 per cent. If a logarithmic function is introduced, the rotation of the Nicol prism should not exceed 55° for $Log_{10} \cos^2\phi$ and 35° for $Log_{10} \cos^4\phi$, to obtain a precision of 0.5 per cent. Similar curves plotted on a larger scale are of great assistance in directly reading the intensity for each scale reading from the curve.

To obtain the precision stated in the preceding paragraph it is necessary to adjust the brightness of the photometric field so that a brightness level ranging from 20 to 30 millilamberts is secured. A more detailed description of the effect of "brightness level" upon the precision obtainable in photometry may be found in Lowry's[1] paper.

In place of the system of Nicol prisms one or more calibrated wedges may be substituted in one or both fields of the photometric system.

A Martens polarization photometer[2] may be substituted for both the system of Nicol prisms and the photometer cube.

III. The cell and test tubes (figures 3, 4 and 5)

The use of test tubes of varying size in the Tyndallmeter-Colorimeter has been made possible by observing a small constant area of the Tyndall cone produced by the scattered-reflected light in the turbid suspension. Figure 3 shows a square tube in cell (C) arranged for a turbidity determination. Screws K1 and K2 and their attached angle bars are for holding the test tube in position. The square tube is the ideal test tube to use for turbidity measurements as it permits the use of a path of light which traverses a very small distance, thereby eliminating many secondary effects which are produced when the path of light traverses a larger distance. Screw K2 is fixed and the size of the test tube will not affect the measurement provided the side of

the square tube is not smaller than the total length of the path of light in the test tube.

Round test tubes (figure 4) may be used in the Tyndallmeter-Colorimeter for turbidity measurements, but the path of light will have to traverse a longer distance if tubes of varying size are to be used. Large round test tubes varying in size as much as 4 mm. internal diameter may be used without correcting for the size of the tube. When Wasserman tubes are used, the permissable variation in size without correction is 1 mm. Figure 4 shows two round test tubes in place and the position of screw K2. By using a small beam of light which traverses a small distance in the test tube, the lens effect of the round tube does not enter into consideration as a variant for different size tubes.
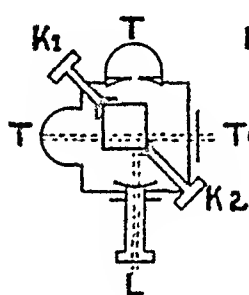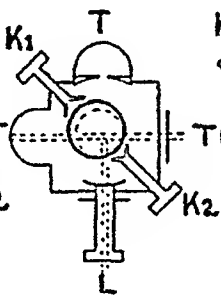

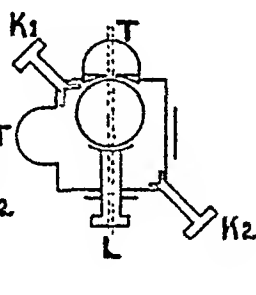FIG. 3   FIG. 4   FIG. 5

Two hollow black tubes (T) sealed at one end are placed opposite each aperture in cell (C) to absorb the transmitted and reflected light.

Figure 5 shows a round test tube arranged for colorimetry. Screws K1 and K2 have been turned back and screw L now holds the test tube in position. The effect upon the measurement produced by using test tubes of different size may be calculated from Lambert's or Beer's laws for the absorption of light by fluids.

SUMMARY

A. Physical advantages of the Tyndallmeter-Colorimeter

(1) The precision of the Tyndallmeter-Colorimeter as experimentally determined by turbidity measurements with the Mar-

tens polarisation or the two Nicol prism photometer is 0.5 per cent.

(2) The turbidity or concentration of suspensions and solutions ranging in concentration from 1 to 10^4 may be determined.

(3) Turbidimetric and colorimetric measurements may be carried out in test tubes without removing the plug protecting the contents of the tube and without loss of any portion of the sample.

(4) Test tubes of varying size may be used in the instrument for these determinations. For turbidity work the size of the test tube will not affect the measurement. For colorimetry, the effect upon the measurement introduced by using test tubes varying in size may be calculated from Lambert's or Beer's laws for the absorption of light by fluids.

(5) One source of radiation (an electric lamp) is used for both the known and unknown substances, thereby automatically compensating for changes in the energy and other factors influencing the intensity or quality of the source of light.

(6) The standard of reference to which the turbidity or color of the unknown suspension or solution is compared is a physical unit which does not change with time nor deteriorate.

B. Applications of the Tyndallmeter-Colorimeter to measurements which are of interest in medicine

(1) The quantitative determination of the turbidity of all turbid substances.

(2) The determination of bacterial concentration in salt solution suspensions, as a function of the size and number of the organisms. Similar determinations may be made on broth cultures, but a correction must be made for the turbidity of the broth and the turbidity produced by the metabolic products of the bacteria.

(3) The determination of the average area of blood corpuscles when the number of corpuscles in a suspension is known. This value is of significance in anemic blood as an index of the average size of the red blood corpuscles if the number of white blood corpuscles is within normal limits.

C. As a colorimeter

(1) All colorimetric determinations such as blood sugar, blood urea, creatinine, and similar laboratory procedures can be performed.

(2) The hemoglobin content of blood can be determined.

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SHOULD THE PRECIPITATION TEST FOR SYPHILIS BE ADOPTED TO THE EXCLUSION OF COMPLEMENT-FIXATION PROCEDURES?*

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The advent of the Kahn precipitation tests for the laboratory diagnosis of syphilis unquestionably marked a new era in the re-valuation of various preëxisting tests for that disease. It has stimulated numerous valuable researches along the lines of "specificity" and "sensitiveness." Through such researches knowledge of the nature of the precipitation and of the complement-fixation reactions as applied to the laboratory diagnosis of syphilis has become considerably enriched. Indeed, the mechanism of these reactions can be expressed now in simple physico-chemical formulas. Investigators who make this subject their special study know what changes they may expect in the results following given changes in the reagents used.

A considerable number of such researches can be found scattered among the scientific and medical publications, while much of this information remains unpublished. Unfortunately many of the practical serologists read the published results entirely too casually, being content with abstracts or with the summaries of the original publications. It is safe to state that the majority of them regard such studies as of purely theoretical importance and are of the opinion that they have no practical significance or direct bearing upon the understanding of certain inconsistencies or discrepancies occurring in the results of the various tests for syphilis.

It is for this reason that the tube-precipitation test, for instance, because of its simplicity of manipulation, apparent definiteness

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of the standardization of antigen, comparative high degree of "sensitiveness" and "specificity" and facility of reading the results, has profoundly impressed the practical serologists in the United States and abroad. It has caused many of them to abolish the complement-fixation procedure and rely upon the results of the precipitation test exclusively. Enthusiasts went so far as to claim for their test superior, if not absolute "sensitiveness" and "specificity" and the attributes of a quantitative science.[2] The sero-luetic reports of the League of Nations Health Committee competitive conferences in Copenhagen and in Montevideo strengthened the impression that the complement-fixation procedures are by nature inferior and less reliable.[3] This despite the fact that at either of these conferences complement-fixation cold incubation procedures based upon the technical principles described by Kolmer[4] were not presented.

The above outlined situation has crystallized itself into a definite pressure upon serologists who still adhere to the use of the two types of procedures. This pressure is directed along several channels. First, there is the constant demand for "while you wait" results; second, there is the argument connected with the reduction in the cost; third, there is the altogether scientifically unjustifiable tendency to refer results of any and all tests to the tube-precipitation test. Even the best informed students of serology upon reporting a negative precipitation and positive complement-fixation (cold incubation) on the same serum specimen have been told that their complement-fixation technic yields nonspecific results, and is, therefore, confusing and useless; while if they report a reverse condition, they are told that their complement-fixation technic is insufficiently sensitive, and, therefore, again confusing and useless.

Serologists must take a definite well-founded stand as to whether one or two types of reactions shall be given preference in the practical serologic laboratory. The following brief discussion of some theoretical and practical aspects of complement-fixation and antigen precipitation is presented to assist in forming a rational opinion on this question.

It is well known that the precipitation test becomes positive

earlier than any complement-fixation test in some instances of early syphilis. On the other hand, I have reported consistently positive complement-fixation results in many instances of early syphilis in which the precipitation test lagged considerably. In this connection the old question arose as to whether the precipitins in the case of syphilis are identical or merely concomitant with the so-called lytic sensitizers. This question is of importance from a practical immunologic view-point. It is evident from the nature of the lytic sensitizers that the answer to this question cannot be attained without the assistance of the lytic agent per se-complement. Complement-fixation as a procedure, therefore, cannot be abolished from the practical serologic laboratory until this question finds its solution.

This argument in favor of complement-fixation may be regarded by many as a weak one. Indeed, if allowed to stand by itself it carries but little persuasion. Combined with the following arguments into a logical system of reasoning, it becomes significant.

From a physicochemical consideration any substance that is in solution is a part of the solution system, and any substance that has become precipitated from the solution is from a practical point of view without the solution system. In the instance of substances in the colloidal state this generalization is equally true, though in a somewhat qualified way. Generally speaking, the chemophysiologic influence of a substance in the colloidal state is determined by its chemical properties. However, many of its characteristics, such as the velocity and intensity of reaction, are determined largely by the enormous surface presented per unit mass. Where a mass-unit of substance is subdivided so that the resulting subdivision approximates "all surface," interfacial phenomena, such as surface tension, adsorption, electrical potential, and solubility become enchanced out of proportion to the mass relationships. Hence, the chemophysiologic reactivity of a substance in a high degree of subdivision may be as great as that of a substance in true solution. It may be regarded, therefore, with justice as within the solution system. Any physicochemical process, which reduces the surface area of the substance in extreme colloidal state to a point where visible pre-

cipitation and settling out appear, reduces its potency to a point nearing inertness. Hence, once the substance originally in colloidal suspension is precipitated, it can be regarded from a practical view-point as without the solution system.

This principle is of importance to the living organism teleologically. A substance which finds its way into the circulating fluid or into the fluids surrounding the tissues may be directly utilizable by the organism in its economy, or it may not be utilizable by the organism in its original state. If it is of the former type and is present in proportionate amount, it is of assistance to the organism in its normal physiologic processes. If, on the other hand, it is of the second type, it will hinder the physiologic processes of the organism and may be considered as toxic. It must be removed from the body.

If the molecules of the interfering substance, present in the body fluids in solution or in colloidal state, are of an organic nature and are such that they can be eliminated through the skin, respiratory or urinary tracts, or can be directly transformed into non-interfering molecules by the liver or other organ of the body, no toxic condition may arise and no immunologic defense reaction may be evoked. If, on the other hand, the molecules of the foreign organic substance cannot be rendered harmless by the means just enumerated, in most cases immunity reactions, as understood by serologists, come into play. The injurious substance must be placed without the solution system of the body as the first and immediate step towards protection. To accomplish such precipitation, the immunoprecipitins are generated by the living organism. These immunobodies undoubtedly serve other purposes with which this discussion is not concerned. The earlier the precipitins appear and the more generous their amount, the greater the body protection.

Once the foreign substance has become precipitated, its immediate injurious effect to the organism is removed. The lytic sensitizers then may come into play and sensitize the substance to the action of complement. The latter, whatever its mechanism of action may be, transforms the original substance, so that the resulting molecules can either be eliminated or utilized by the organism.

If this teleologic theory of the process of immunity is accepted, then it must be assumed that precipitins appear first, and, therefore, can be first demonstrated. It is possible that this may well answer the question as to why in a large percentage of cases of beginning syphilis positive precipitation results are obtainable comparatively early. It may constitute a rational scientific reason for the use of the precipitation test for syphilis. It cannot, however, be regarded as an argument in favor of the abolition of the complement-fixation tests, since other factors arising from the quantitative relationship of the immunologic principles must be considered.

The well known adage of practical medicine "every case is a law unto itself" is applicable to immunologic manifestations in vivo and in vitro. Unquestionably, this is largely responsible for the failures in generalized therapeutic immunology. The factors entering into play are numerous and variable, and at the present stage of our knowledge many of them are unaccountable. If we assume, according to the above outlined theory, that the precipitins and sensitizing lysins constitute two distinct factors and that the precipitins, as a matter of immediate protection, are generated by the living organism in advance of the sensitizing lysins, we still remain in ignorance with regard to the progress of their generation in any individual case.

What, for instance, would be the effect upon the results of antigen precipitation and complement-fixation in a case in which the generation of the precipitin is slow, though early, and that of the lytic sensitizer is normal or accelerated? Here, the factors of dispersion, of reactive surface area of the suspended particles, and the manner in which complement combines with the sensitized suspension, offer a possible explanation. The recording of the tube-precipitation results, it must be remembered, depends upon ocular observation. But ocular visibility has its limits even with the aid of a magnifying glass. Where the concentration of the precipitin is low, high dispersion will result. High dispersion means an increased total surface area of the particles suspended. With the normal or accelerated rate of generation of the lytic sensitizer, enough of it will be present to sensitize the entire surface area of the highly dispersed suspensoid.

It is now generally conceded, in fact it is nigh well proven, that complement acts through the process of adhesion by coating the surface area of the sensitized particles in the suspensoid in a layer of definite effective thickness.[15] The greater the surface area of the sensitized substance, the greater the consumption of complement. Hence from a practical laboratory viewpoint in the above hypothetical case there is a comparatively small total volume of an immunologically sensitized substance, so highly dispersed as to be invisible to the eye alone or with the aid of a magnifying glass. The result, therefore, must be reported as negative by the usual tube-precipitation method. Though beyond the visibility of the eye, the suspensoid represents a truly immunologically sensitized complex, since the quantity of lytic sensitizer was assumed to be generated at a progressively greater rate than the precipitin. It is, therefore, capable of adsorbing complement according to the accepted principles of immunology. This substance, being highly dispersed and, thereby, forming a large surface area, is capable of fixing a complete dose, or more, of complement. The result is a four plus complement-fixation, in the presence of a negative precipitation.

From the antigen-precipitation negative to the doubtful is only a short step. The Kahn doubtful, according to the author of the test, is to be reported as negative. Yet, the occurrence of serum specimens giving results of Kahn tests which for research record purposes were marked as "doubtful-trace in one or two tubes" and which resulted in strongly positive complement fixation tests have not been infrequent in my experience. True enough, they occur very infrequently in cases of early syphilis and most frequently in treated cases. But in this very fact I see a possible danger. In the absence of a better criterion for the treatment of syphilis, many syphilologists have been guided by the laboratory results. Much, of course, has been said and written to condemn such a practice. Since, however, nothing even approaching a positive substitute has been offered, the practice has been continued by some of our best syphilologists. The use of both the precipitation and complement-fixation tests eliminates the possibility of making the undesirable impression upon the

clinician. By receiving precipitation negative and complement-fixation positive reports, the clinician will have his attention directed to the fact that antibody is still detectable by one of the tests used. Thereby, the laboratory will have fulfilled its function.

Further theoretical considerations of the mechanism of the tube-precipitation test and experimental evidence strengthen the objection to the elimination of the complement-fixation procedure from the practical laboratory. It was shown by many investigators[8] that up to the addition of the hemolytic system, the complement-fixation and antigen-precipitation tests are effected by the same immunochemical mechanism. However, in the first case, an indicator must be added to make the end result evident, while in the latter the end result becomes directly visible to a varying degree. Such visibility is brought about by purely mechanical means, based upon certain physicochemical properties inherent to the colloidal suspensions primarily concerned in the tests.

Upon the addition of the serum to the antigenic suspension employed in either the antigen-precipitation or the complement-fixation procedures, active suspended nuclei are formed. A complexity of interfacial forces arises at the surfaces of these nuclei. A particular combination of these forces exerts a selective attraction upon the precipitins and lytic sensitizers. Through the process of adsorption the latter coat the suspended particles in a successive order and become denaturized through the process of dehydration, polymerization, or the like.[9] At the same time the difference between the surface tension of the medium and the suspended particles of the antigen is altered. The surface tension of the new sensitized antigenic particles in particular acquires an optimal value which makes the discrete particles susceptible to complete or partial aggregation and coalescence.

The suspended particles are in constant molecular motion and frequently collide. If the frequency of the collision and the impact force are great enough to overcome the resultant of the forces of dispersion, the sensitized particles will aggregate and coalesce forming progressively increasing spheres or spheroids. At

a certain point the particles become too great and too massive for the Brownian movement. Further collision and coalescence will cease. It follows, that the greater the concentration of the suspensoid, up to a proper colloidal balance, the greater is the opportunity for the particles to collide and coalesce; also, the greater the concentration of the sensitizer, the greater the favorable change in the surface tension, and, hence, less impact force is required to effect coalescence. Other factors, such as the influence of the concentration and of the type of electrolyte present undoubtedly are of equal importance.

In the case of the complement-fixation technic all the reagents are used in high dilution. The number of spheres or spheroids per unit volume is comparatively small, the distance between the particles is comparatively great, while the amount of precipitin and, hence, the reduction in the surface area and the optimal change in the surface tension of the particles are comparatively small. It is evident, therefore, from a simple consideration of dynamics, why the particles should remain invisible and why the indicator should be necessary to make the end result demonstrable.

In the case of the precipitation test all the reagents are used in the greatest possible concentration, and the final volume is kept at a minimum. This makes the distance between the particles short. The particles per unit volume are numerous. The changes in the surface tension difference and in the surface tension of the sensitized particles per se are shifted to magnitudes more favorable than in the case of the reagents used in the complement-fixation test. This leads to an increase in the frequency of collision between the particles and to a greater susceptibility on their part to coalescence. It makes the same impact force, due to molecular movement of the particles, more effective in causing the aggregation and coalescence of the sensitized particles. Hence, the visibility of the results of the tube-precipitation test.

However, even in the Kahn test the particles soon reach a size too large for the force supplied by the Brownian movement. Depending upon the balance of forces, aggregation and coalescence of the particles stop when the so-called precipitate is

barely or not at all visible in many positive serums. An outside force must be adopted; hence, the shaking process which continues with greater force and greater effectiveness, the work started by the Brownian movement of the particles. The process is apparently similar to that of churning, but too forceful and too long continued shaking may reëmulsify the aggregated and coalesced particles in some positive serums. Therefore, the time of shaking and the number of agitations per minute must be prescribed.

This definitely prescribed time and manner of shaking is an essential factor from a practical consideration. But it carries with it its own weakness, and presents further argument against the adoption of any precipitation test to the complete exclusion of a well standardized cold incubation complement-fixation test. As in clinical medicine "every case is a law unto itself," so in serology "each serum has its own idiosyncracies." The force supplied by the shaking process may be sufficient to bring about maximal visibility in one specimen, it may be insufficient for another, and may be too much, and hence, have a dispersive effect on still another. From a practical laboratory consideration, therefore, it is evident why some Kahn tests should read negative or doubtful (reportable negative), yet the complement-fixation tests should be positive in varying degree. This has occurred repeatedly in my experience with serums from individuals with definite histories of syphilis. The fact that some of them may be treated patients, or patients who have received maximal treatment, or patients who may be considered by some syphilologists as cured, matters not so far as the thesis of this paper is concerned. No agreement exists among the best syphilologists upon the definition of cured or even arrested syphilis; nor is it definitely established whether the antibody demonstrable in a long treated case of syphilis is an indication of continued immunity or of a feebly continued or reawakened activity of a host. What matters, indeed, is the proper decision as to whether serologists should rely upon ocular visibility of precipitation tests alone as a scientific basis for their serologic judgment, setting aside once and for all complement-fixation procedures as a

practical laboratory application. In the light of this discussion, the answer is unequivocally negative.

EXPERIMENTAL

If the shaking process used in the Kahn test is insufficient for some serums and redispersive for others, cannot an applicable mechanical force be resorted to which would prove more general in its efficacy as a coalescing agent? Indeed, such a force is supplied by centrifugation. It has been employed by investigators previously and by myself in some of my[7] theoretical studies. Recently Mueller[10] applied it to his laboratory test for syphilis. The following experiments prove that some Kahn negative and doubtful tests contain immunologically sensitized reagin in an invisible state of division.

One series of cholesterolized and one of Kolmer lecitholized antigen complement-fixation tests and two sets of Kahn tests were prepared on a number of the same serums. One Kahn series was completed, according to Kahn routine, using the second and third tubes only.[6] The results were recorded for each tube. The duplicate series was placed in the incubator for one hour, following the shaking. At the end of one hour and prior to the addition of the saline the tubes were centrifugalized for ten minutes at a speed exceeding 3000 revolutions per minute. One half cubic centimeter of saline was then added to each tube. According to the completeness with which the antigen became removed from suspension and the degree of coalescence of the suspended particles, the results were recorded as usual, $++++$, $+++$, $++$, $+$, and doubtful. This experimental procedure was applied to one thousand random specimens. Some of the results which have a direct bearing on the subject under discussion are tabulated.

The cholesterolized and the Kolmer are three-tube cold incubation tests. In the table the control tubes are not reported, since tests with anticomplementary results were omitted. After recording the results of each tube, the Kahn-experimental series were thoroughly shaken and carried through a complement-fixation procedure as described by me[7] in another paper.[8] In every case the results were positive. An equal number of confirmed negative tests were subjected to a similar procedure. The complement-fixation results in every case were negative. The presence of a highly dispersed sensitized reagin, invisible to the standard Kahn test has been demonstrated by this experiment in every case where one or both of the complement-fixation tests were positive. The significance of the few cases in which all the routine tests were negative and the post-centrifugalized positive has no direct bearing on the subject under discussion and will be discussed elsewhere.

The results of the tests recorded in the table were taken at random from the research notebook. No information was appended to the records heaving upon the clinical histories of the cases. It might have been suspected, therefore, that in some of the cases there was no history of syphilis. Were it so, the results

TABLE 1

Comparison of Results by Different Methods

NUMBER	CHOLESTEROLIZED	KOLMER	EARN	EXPERIMENTAL
1	2.1	1.1	0.0	2.3
2	4.4	3.1	Tr. Tr.	2.3
3	4.4	4.4	Tr. Tr.	3.3
4	3.1	0.0	0.0	0.0
5	4.2	3.2	0. Tr.	1.2
6	0.0	0.0	0.0	1.1
7	2.1	0.0	Tr. Tr.	2.3
8	3.2	1.0	Tr. Tr.	3.4
9	3.2	2.0	0.0	4.4
10	2.1	0.0	0.0	3.4
11	4.4	0.0	Tr. Tr.	4.4
12	0.0	0.0	0.0	2.2
13	4.4	1.1	0.0	1.2
14	2.1	1.1	0.1	4.4
15	4.4	3.3	0.0	2.2
16	3.2	0.0	0.0	2.3
17	3.3	3.3	0.0	1.1
18	4.4	4.4	0.0	1.0
19	0.0	0.0	0.0	3.4
20	3.3	3.2	0.0	2.2
21	3.1	1.0	0.0	4.4
22	3.3	2.2	0.0	2.2
23	3.3	2.1	0.0	3.4
24	4.4	4.4	0. Tr.	1.1
25	2.1	1.1	0.0	3.3
26	1.0	2.1	0.0	2.2

of the experiment would be valueless. However, after the table has been compiled, excerpts were made from the clinical records of the cases. In each there was a history of syphilis. Some of the excerpts are presented below:

(1) In June 1930, the patient observed an ulcer on the vulva which persisted several days. July 31, 1930, blood Wassermann (type not described) was ++++. Had received treatment. Admission Wassermann (warm incuba-

tion) Negative: Kahn, $+++$. Diagnosis: Syphilis, secondary, recurrent. Patient is under treatment. History of serology: July 2, 1931, Cholesterolized and Kolmer, Doubtful; Kahn, $+$; August 23, 1931, all tests $++++$; December 11, 1931, as shown in table.

(2) Patient complains of vaginal discharge and pain in lower abdomen. History of miscarriage, claimed to be accidental. States blood tests taken one year prior to this examination was Negative. Upon admission serologic tests were: Cholesterolized, $++$; Kolmer, $++$; Kahn, $+$. December 11, Cholesterolized, $+++$; Kolmer, $++$; Kahn, Negative.

(3) Diagnosis: Syphilis, latent. Feb. 5, 1929, Wassermann (warm incubation) and Kahn, Negative. Several months later, Wassermann, and Kahn, $+++$. September 3, 1931, Wassermann and Kahn, Negative. December 11, 1931, Cholesterolized, $+++$; Kolmer, $++$; Kahn, Negative.

(4) Diagnosis: Syphilis, primary. History of serology: March 30, 1931, Wassermann (warm incubation) Negative; Kahn, $++++$. August 7, 1931, Cholesterolized, $++++$; Kolmer and Kahn, $+$. December 7, 1931, all tests Negative.

(5) Syphilis, secondary. History of treatment.

(6) Had genital lesion twenty years ago. Antisyphilitic treatment one year ago. Serology vascillated throughout the period of observation from Negative, to strongly Positive, then Doubtful, and again strongly Positive, the Kahn test being Negative most of the time. December 11, all routine tests were Negative, while experimental test was $++++$.

(7) Had sore on penis two years ago. Spinal tests all positive. Diagnosis: Neurosyphilis. It is worth observing that in this case on December 7, 1931, the serologic reports were: Cholesterolized, $++++$; Kolmer, $++++$; Kahn, Negative; Experimental, $+$.

(8) Diagnosis: Syphilis, latent. Contracted eight months ago. Only complaint upon examination: itching of skin. From March 23, 1931 to November 2, 1931, serology was consistently Negative. On that date, Cholesterolized, Kolmer, and Kahn, $++$. November 9, 1931, Cholesterolized, $++$; Kahn and Kolmer, Doubtful. December 10, 1931, all tests were Negative, while experimental was $++$.

(9) Patient was referred by Mexican Health Center with positive serology. Diagnosis: Syphilis, Latent.

(10) Patient is five months pregnant. Three weeks prior to presentation at Clinic had Kahn, $++++$. Is free from clinical symptoms. Upon examination Cholesterolized, Kolmer and Kahn were $++++$. September 28, 1931, all tests were again $++++$. December 7, 1931, Cholesterolized, $++$; Kolmer, Negative; Kahn, Faint trace; experimental, $++$.

(11) Had syphilis in 1920 at which time patient received treatment. Has been free from clinical symptoms for six years, and none were observable at the time of presentation at the clinic. But serologic tests were: Wassermann (warm incubation), $++$; Kahn, $++++$. May 18, 1931, Wassermann (warm

incubation), $+++$; Kahn, $++++$. August 25, 1931, Cholesterolized, $++$; Kahn, $++++$. December 7, 1931, Cholesterolized, $++$; Kolmer, Doubtful; Kahn, Faint trace; Experimental, $++++$.

(12) Diagnosis: Syphilis, latent, neurosyphilis. Serology varied from fixation positive, precipitation negative, to reverse. Cerebrospinal fluid tests, Positive.

The twelve excerpts are like the remaining fourteen. The points of importance brought out by excerpts are: (1) In treated cases of syphilis both types of tests show periods of nonreaction. In most cases these periods are not coincidental. (2) No parallelism exists between the results of the precipitation or complement-fixation tests and the clinical symptoms. (3) The serologic results of either of the two types of tests follow no well defined curve of intensity-reduction in the course of treatment. (4) Where the standard Kahn is negative in treated cases, the lecitholised Kolmer is frequently positive, (the reverse is also true to a great extent, but we are not concerned with this in the present paper), the cholesterolized is generally positive, and the experimental centrifugalization results are nearly always positive. The results of the experiments, as summarized in the preceding table, supported by the information supplied by the excerpts from the histories, strengthen the thesis of this paper and offer the concluding argument against the abolition of the standardized complement-fixation procedures from the diagnostic laboratory and the adoption of the precipitation test as the sole demonstrator of the presence of reactive substance in syphilitic serum.

SUMMARY

Definite pressure has been brought to bear upon workers in the field of serology in syphilis to eliminate the complement-fixation procedure from the diagnostic laboratory, and to limit the serologic diagnosis of syphilis to the tube-precipitation test. To assist serologists and laboratory technologists in deciding upon the stand to be taken in relation to such pressure, a general discussion of the principles upon which precipitation and complement-fixation tests are based is presented. The bearing which the dualistic conception of the origin of luetic precipitins and lytic

sensitizers may have upon the interpretation of the discrepancies occurring between the results of the precipitation and complement-fixation results is also discussed. The term lytic sensitizers instead of lysins is here used advisedly. A teleologic theory of the functions of the precipitins and lytic sensitizers is presented. In the light of this theory it is assumed that precipitins are generated in advance of the lytic sensitizers. It is further assumed that the progress of generating precipitins may be less, parallel to, or greater than that of the lytic sensitizers. Conjointly with the mode of the complement function such progress variation, it is pointed out, may have a direct bearing upon the differences in the results of antigen precipitation and complement-fixation. Experimental evidence is presented to prove on a practical basis the points brought out in the discussion of the physicochemical factors which in the same serum may be favorable to a strong complement fixation and unfavorable to the tube-precipitation test. It is concluded that nonvisibility of the precipitate in the tube-test does not constitute a scientific criterion for the judgment as to the absence of the immunologically sensitized antigen-antibody complex. A well standardized and properly carried out complement-fixation test demonstrates the presence of sensitized complexes in most positive serums, no matter how high the degree of their subdivision may be. It is, therefore, concluded that the complement-fixation, including overnight icebox incubation and the tube-precipitation tests, must be continued as mutually supplementary adjunct procedures to the diagnosis and control of syphilis.

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VALUE OF H AND O AGGLUTINATION IN DIAGNOSIS OF TYPHOID*

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Since the advent of prophylactic vaccination against typhoid fever, the interpretation of agglutination reactions has been confusing. This is due to the fact that the serum of inoculated persons will agglutinate the organism in question. In view of this fact Dreyer[2] introduced his method of repeated estimation of the agglutinin content of the patient's serum. However, in the case of *Salmonella paratyphi* (*B. paratyphosus A*), the agglutinin response may be so slight as to render the repeated estimations uncertain. Recently, Felix[3] published evidence to show that protective inoculations lead to the formation of large flaking agglutinins, while the agglutination in typhoid fever and in a non-inoculated person is, without exception, of the small flake type.

The explanation of the small and large flaking forms is found in the work of Weil and Felix[?] who showed that Proteus X exists in two forms (O and H forms), which differ from each other morphologically, biologically and serologically. They represent two forms of variation mutually transferable into each other. The anti-O-serum contains one agglutinin which reacts specifically with the homologous organisms agglutinating them in small flakes (O-agglutination); antiserum against the H type contains two agglutinins, the specific small flaking O and a nonspecific large flaking H which reacts with heterologous as well as with homologous organisms. The O receptors are thermostabile while the H receptors are thermolabile (Sachs).[8]

Burnet[1] believed that the O types are due to the somatic proteins, and the H type to the flagellar proteins. Accordingly,

in a given case of typhoid fever there is an overwhelming stimulation of the O or small flaking agglutinins, while in the inoculated person the H type or large flaking agglutinin predominates. In a series of eighty-seven individuals given antityphoid inoculations, Gardner[5] observed the production of the O form, and pointed out that it is likely inoculation does not cause, on an average, nearly so great a rise of the O forms as does typhoid fever, and therefore, the two conditions are not readily distinguishable. The O titre is higher shortly after inoculations than after a long period has past.

The question of a difference in the type of agglutination produced between the serum of a person actively infected and one previously inoculated is not a closed one. The recent outbreak of typhoid fever in the Cleveland State Hospital for the insane afforded an opportunity to investigate this problem further.

TECHNIC

The method of Felix was followed in detail. The strain of typhoid bacillus was the Kinyoun strain of the Ohio State Department of Health. The organism does not self-agglutinate. Twenty-four hour growths on plain agar were washed off with 1.5 to 3 cc. of saline depending on the size of the slant, and one drop of the suspension was added to each tube of serum dilution (1 cc.). The following dilutions were used: 1:50, 1:100, 1:250, 1:500, 1:1000 and 1:2000, and the usual saline organism controls. The tubes were incubated at 37°C. for two hours and sixteen hours at room temperature. All observations were made with a magnifying glass and readings were also made at the end of forty-eight hours. Both inactivated and active sera were also employed.

RESULTS

In a series of forty-two typical cases of typhoid fever it was found after eighteen hours that in a 1:50 dilution 90.2 per cent showed small flakes and 7.3 per cent large flakes. At the end of forty-eight hours the percentage showing the small flake type dropped to 70.7 per cent, while the large flake type increased 26.8 per cent. (See table 1.)

In the 1:100 dilution at eighteen hours the small flaked agglutination was 80.5 per cent, dropping to 75.6 per cent in forty-

eight hours, while the large flake type increased from 9.7 per cent to 17.1 per cent.

In a series of forty inoculated individuals including six treated intravenously for shock therapy, seven vaccinated from sixteen to eighteen months before, four vaccinated five months previously, and twenty-three from four to six weeks before, it was observed at eighteen hours that in a dilution 1:50, 55 per cent of the serums showed small flakes and 37.5 per cent large flakes.

TABLE 1

THE PERCENTAGE OF SMALL AND LARGE FLAKE AGGLUTINATIONS WITH SERUMS OF CASES OF TYPHOID FEVER

ACTIVE CASES		SMALL	LARGE	MIXED	NEGATIVE
dilution	hours incubated	per cent	per cent	per cent	per cent
1:50	18	90.2	7.3	0	2.4
	48	70.7	26.8	2.4	0
1:100	18	80.5	9.7	0	9.7
	48	75.6	17.1	2.4	4.9

TABLE 2

THE PERCENTAGE OF SMALL AND LARGE FLAKE AGGLUTINATIONS WITH SERUMS OF INOCULATED PERSONS

IMMUNE CASES		SMALL	LARGE	MIXED	NEGATIVE
dilution	hours incubated	per cent	per cent	per cent	per cent
1:50	18	55.0	37.5	2.5	5.0
	48	40.0	52.5	2.5	5.0
1:100	18	42.5	42.5	0	15.0
	48	47.5	40.0	2.5	10.0

In forty-eight hours the ratio had changed to 40.0 per cent for the small flake type and 52.5 per cent for the large flake type. At eighteen hours in the 1:100 dilution 42.5 per cent were of the small and 42.5 per cent of the large flake type. In forty-eight hours 47.5 per cent showed small and 40.0 per cent large flakes. (See table 2.) This ratio was not appreciably different between those vaccinated recently and those vaccinated eighteen months previously, although the serums of those individuals vaccinated recently showed agglutination in higher titers.

Although it is thus shown that 40 to 50 per cent of the serums of inoculated individuals agglutinated in small flakes when classed as to size, in many of these cases the floccules appeared to be less compact and more loosely scattered than those of the same size in the serums of patients with typhoid fever, in which the agglutination appeared more compact and granular in type.

Felix believed that it was necessary to use only a single serum dilution and suggested the 1:100 dilution. We found, however, that a single dilution could not be relied upon for representative results. The higher dilutions used by us showed nothing of importance. Gardner[5] used both 1:100 and 1:400 dilutions.

Our results demonstrate that while there is a predominance of O-agglutination in the serums of those actively infected with typhoid there is also O-agglutination in about 50 per cent of those inoculated against the disease, regardless of time whether recently or long before.

Felix and Olitzki[6] pointed out that with *Eberthella typhi* (B. typhosus), *Salmonella paratyphi*, *Salmonella schotmülleri* (B. paratyphosus B), and *Salmonella enteritidis* (B. enteritidis), low concentrations of phenol and formol produced definite inhibition of O (small flake) agglutination, while the H (large flake) type was unaltered. Alcohol in low concentrations seemed to produce no such inhibitory effect, but in high concentrations alcohol inhibited the H type of flocculation. In a series of ten serums from inoculated persons and fifteen serums from active cases the agglutination with live organisms was compared with that produced in bacterial suspensions made with 0.1 per cent formol, and 5 and 50 per cent alcohol. With the formolized suspensions there was 70 to 100 per cent H flocculation, the average being 76.6 per cent (see table 3). With 5 per cent alcohol suspension of bacteria there was also a predominance of the H type, 20 to 90 per cent (see table 3), although with a much lower average (60.0 per cent). Fifty per cent alcohol completely inhibited the H agglutination, there being about 50 per cent 0 and 50 per cent negative results (see table 3). This bears out Felix' contention that the use of live bacteria is essential for a clear differentiation.

THE "ANAMNESTIC REACTION"

Krauss and Barrera[6] reported two cases of a positive Weil-Felix reaction (Proteus OX$_{19}$) in a case of typhoid and a case of measles. In the case of typhoid the Weil-Felix was positive

TABLE 3

INCIDENCE OF DIFFERENT TYPES OF AGGLUTINATION OF TYPHOID BACILLI, SHOWING THE EFFECT OF FORMOL AND ALCOHOL

TEST		LIVE ORGANISMS			0.1 PER CENT FORMALIN			5 PER CENT ALCOHOL			50 PER CENT ALCOHOL		
		Small	Large	Negative	Small	Large	Negative	Small	Large	Negative	Small	Large	Negative
(1) Serums of fifteen active cases													
dilution	hours incubation	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
1:50	18	80	20	0	20	80	0	10	90	0	20	0	80
	48	70	30	0	0	100	0	10	90	0	30	0	70
1:100	18	70	30	0	70	30	0	60	40	0	10	0	90
	48	70	30	0	30	70	0	60	40	0	10	0	90
(2) Serums of ten inoculated individuals													
1:50	18	93	0	7	20	80	0	20	80	0	53	0	47
	48	67	33	0	20	80	0	40	60	0	53	0	47
1:100	18	73	7	20	20	80	0	40	60	0	20	0	80
	48	67	20	13	7	93	0	80	20	0	33	0	67

TABLE 4

SHOWING AGGLUTINATION WITH PROTEUS OX$_{19}$

AGGLUTINATION WITH OX$_{19}$		IMMUNE	ACTIVE
		per cent	per cent
2 hours incubation	Positive	57.1	78.9
	Negative	42.8	21.0
18 hours incubation	Positive	100.0	100.0

before a positive Widal of 1:4000 was obtained. Both patients lived in Buenos Aires where typhus fever is not known. However, one came from Russia and one from Ireland where endemic

typhus fever exists.   More recently Palacios and Armijo[7] obtained positive Weil-Felix reactions in twelve cases of typhoid fever.   The reaction was positive in dilutions of from 100 to 200 but in Chile, typhus fever is endemic.

We tested all serums from both the active cases of typhoid fever and the inoculated individuals using dilutions of 1:10, 1:20, 1:40, 1:80, 1:160 and 1:320.   After two hours at 37°C., the serums from the inoculated persons agglutinated (O type) in 57.1 per cent of the tubes, and no agglutination was seen in 42.8 per cent (see table 4).   The active typhoid cases showed O type agglutinations in 78.9 per cent and no agglutination in 21.0 per cent.   After standing at room temperature for an additional eighteen hours all the serum agglutinated.   All the serum agglutinated in dilutions up to but not higher than 1:80.   We believe that the reaction should be interpreted on the basis of nonspecific stimulation.

CONCLUSIONS

(1) Agglutination reactions were performed by the method of Felix with the serums of forty persons inoculated against typhoid fever and with the serums of forty-two patients with typhoid fever.

(2) Large flaked or H type agglutinations occurred in 40 to 52.5 per cent of serums of inoculated individuals.   The small flaked or O type was noted in 70 to 90 per cent of the serums from typical cases of typhoid.

(3) While the H type of agglutination, in most instances, would point to inoculation as the cause, the O type of agglutination cannot be relied upon to designate active typhoid infection.

(4) Formalin, 0.1 per cent, inhibits the O type to a large extent, and 5 per cent alcohol to a lesser extent.

(5) A Weil-Felix reaction was present in the cases studied as evidenced by agglutination of the Proteus OX_{19}.

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SERODIAGNOSIS OF MALIGNANT DISEASE

PRELIMINARY REPORT

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Numerous attempts have been made to develop a satisfactory test for malignancy using blood serum. These attempts have been made along three principle lines, namely, chemical, complement-fixation tests, and precipitation reactions. As new as the subject of diagnosis of malignancy by a study of the blood may seem, a full review of all the methods advocated would in itself be of burdensome length. Studies upon this subject were begun about twenty years ago by M. Ascoli of Italy. Of the chemical tests may be mentioned the meiostagmin reaction of Ascoli and Izar, the work of Freund-Kaminer, Ruffo, Bothello, Kahn (Germany) Abderhalden, Shaw-Mackenzie, Fuchs and others. All of these have proved unsatisfactory in other hands.

After an extensive investigation, Fry[1] devised a flocculation test for cancer which he used extensively in the Cancer Research Hospital, London. Landau started his studies on a precipitation test for malignancy in 1928 and in a series of 767 demonstrated carcinoma cases, 75.3 per cent gave a positive reaction, and in 826 proved cases of sarcoma, 75.5 per cent gave a positive result.

L. Hirszfeld and W. Halber[2] have described a complement-fixation test for malignancy.

In 1929 one of us demonstrated a certain affinity of an alcoholic extract of carcinomatous tissue for the serum of patients having malignant tumors. After modifications of our technique, a series of nearly three hundred cases has given results which are sufficiently encouraging to warrant further intensive study. While complete accuracy has not been demonstrated, it must be

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borne in mind that none of the serological tests for syphilis, now twenty-five years old, is absolutely accurate.

METHOD OF PROCEDURE

(1) The serum is drawn in the same way as for any other serological test. Only raw serum is used which is kept in the icebox at 8 to 10°C.

(2) For an antigen, the tissue from a malignant tumor, removed surgically or at autopsy, is dissected carefully free from all fat and normal tissue and ground. To every gram of ground moist tissue so prepared 4 to 10 cc. of 95 per cent alcohol is added. If the quantity of tissue permits, several proportions of tissue to alcohol are used. This mixture should stand eight to ten days in the incubator or icebox. Good results have been obtained from both ways. Not
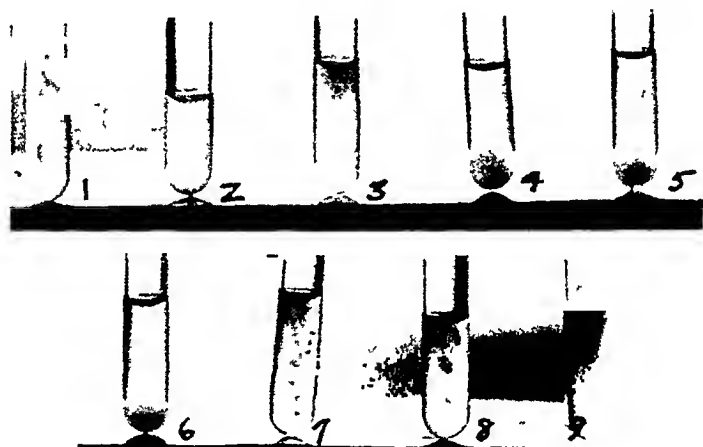

Fig. 1. Tubes 1. 2 and 3 Show Negative Tests; Tubes 4, 5 and 6 Show Positive Tests; Tubes 7, 8 and 9 Show Negative Tests with Clumps

every tissue, however, produces a good antigen in all extract proportions and each one must be tried and titrated with positive and negative controls, and may have to be discarded.

(3) Nine tenths per cent saline is used.

(4) Two hundredths cubic centimeters of raw serum is pipetted into tubes 8 by 50 mm. To the serum 0.5 cc. saline is added, the test-tube rack shaken and 0.1 cc. to 0.2 cc. antigen (depending on the titration) is added. Again the rack is shaken and placed in the icebox at about 8 to 10°C. for four to six hours, sometimes longer. It is desirable to have five controls, negative and positive. The reaction must be watched until the control positives (see fig. 1, tubes 4, 5, 6) show a sedimentation with clear supernatant fluid. The negative tubes are cloudy and colloidal (see fig. 1, tubes 1, 2. 3) without sediment. Sometimes one can see in the negatives suspended clumps, (see fig. 1, tubes 7, 8. 9).

Table 1 shows the results so far obtained.

We are unable to explain why all cancer cases did not give a positive serological test. It must be remembered that some of these malignant neoplasms were young and small, such as carcinomas of lip and cervix and these possibly had not yet become a sufficiently systemic disease as to produce reactive substances in

TABLE 1

	CASES	POSITIVE		DOUBTFUL		NEGATIVE	
		Cases	Per cent	Cases	Per cent	Cases	Per cent
Blood taken preoperatively, diagnosis of malignancy confirmed by microscopical examination	43	38	88.3	1	2.3	4	9.3
Blood taken postoperatively, diagnosis of malignancy confirmed by microscopical examination	33	17	51.0	10	30.3	6	18.1
Clinical diagnosis of no malignancy; confirmed by microscopical examination	28	3	10.0	6	21.4	19	67.8
Clinical diagnosis of malignancy; no microscopical examination	126	92	82.8	11	4.5	23	12.6
Malignancy suspected but not definitely proved	59	17	28.8	7	11.8	35	59.0
Diagnosis: Normal pregnancy	70	4	5.9	8	11.9	58	82.0
Diagnosis: Tuberculosis	23	8	34.7	4	17.3	11	47.8
Diagnosis: Serologically positive syphilis	13	2	15.3	4	30.7	7	53.8

the blood stream. In postoperative cases, only 51 per cent gave positive reactions. A similar observation was made by Volkman.[3]

Syphilitic serums gave 15 per cent positive reactions. Landsteiner and his coworkers showed (1907) that extracts from all normal tissues gave positive results with syphilitic serums. In dissecting malignant tumors, it is impossible to remove all traces of normal tissue since a certain amount of fairly normal stroma is produced in any tumor growth and also bits of normal tissue are found in any area which a malignant tumor is invading or replacing.

Tuberculosis gave 24 per cent positive reactions. At the present time no satisfactory explanation can be given for this fact. It can only be surmised that a similar reactive substance is produced in the blood in both conditions. E. Witebsky[4] said that the right to speak about a specific serologic change in cancer is only possible if the control serums, tuberculosis and pregnancy, do not give a positive reaction. But still he thought there were signs that spoke for specific changes in cancer.

In normal pregnancy, 6 per cent gave positive reactions. It is to be remembered, however, that in this condition a false positive syphilitic reaction is occasionally found. One worker found that a few serums from pregnant women gave a positive complement-fixation reaction with alcohol as an antigen.

SUMMARY AND CONCLUSION

A certain affinity between an extract of malignant tumor tissue and the serum of patients having malignant tumors produces reactions.

We have presented a test for malignancy which in our hands gives about 90 per cent accuracy in cases with malignant tumors. The occurrence of positive reactions in tuberculosis, syphilis and in pregnant patients although not satisfactorily explained, does not seriously interfere with the diagnostic purposes in this test.

A large volume of material with good clinical histories and pathological control would bring this test to a higher degree of accuracy and help to reduce the unspecific reactions.

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TENTH-NORMAL HYDROCHLORIC ACID AS A DIL-UENT FOR COUNTING LEUKOCYTES AFTER INFUSION OF SOLUTION OF ACACIA*

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During the World War, when solution of acacia was first used extensively, it was noticed that the leukocytes often could not be counted in the first three or four days after its infusion. More recently, Huffman[1] thought this might be caused by interference from acacia precipitated in the counting chamber. My attention was first called to the phenomenon by the laboratory technicians of The Mayo Clinic, who were able to recognize that a patient had received acacia by the appearance of the cells in the blood-counting chamber; in attempts to count the leukocytes, the microscopic field was crowded with cells.

When I attempted to count the leukocytes of a patient to whom acacia had been administered recently, using the ordinary diluent (1 per cent acetic acid), a preponderance of unhemolyzed erythrocytes was seen. This failure of hemolysis could be observed grossly, the liquid in the diluting pipette remaining cloudy. It occurred to me that perhaps a stronger acid would hemolyze the erythrocytes without destroying the leukocytes, and hence be suitable as diluent. Tenth-normal hydrochloric acid was available, and I found it apparently fulfilled these qualifications. In order to determine the accuracy of the leukocyte count when tenth-normal hydrochloric acid was used as a diluent, two counts were made on each of seventeen persons who had not been given acacia; 1 per cent acetic acid was used in one pipette and tenth-

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normal hydrochloric acid in another. In each case by statistical methods the count made with hydrochloric acid was as near like that made with the standard diluent as another count would have been if acetic acid had been used in the second pipettes.

TABLE 1

Leukocytes, with Different Diluents, of Patients Who Had Been Given Infusions of Solution of Acacia

CASE	WEIGHT	SOLUTION OF ACACIA INJECTED	DAYS AFTER INFUSION OF ACACIA	LEUKOCYTES IN EACH CUBIC MILLIMETER OF BLOOD, USING AS DILUENTS:	
				1 per cent acetic acid	Tenth-normal hydrochloric acid
	kgm.	*cc.*			
1	63	300	2	Indistinguishable	11,700
2	71	500	2	Indistinguishable	11,500
			4	6,800	
3	77	550	2	Indistinguishable	14,200
			5	Indistinguishable	11,800
4	74	700	2	Indistinguishable	13,200
			3	Indistinguishable	11,700
			5	7,200	
5		700	1	Indistinguishable	13,200
			2	Indistinguishable	9,900
			3	11,900	
6	77	700	1	Indistinguishable	6,200
			2	Indistinguishable	6,200
			3	Indistinguishable	7,400
			4	10,600	10,700
7		1,000	1	Indistinguishable	25,500
			2	Indistinguishable	18,400
			3	Indistinguishable	25,900
			5	15,550	13,950
8		400	2	Indistinguishable	16,700
			3	Indistinguishable	9,500
			5	Indistinguishable	7,600
			8	11,300	11,100

As the opportunity presented, the leukocytes of patients who had been given infusions of 6 per cent solution of acacia were counted; when possible, the first count was made within four hours after infusion. In some instances, the leukocytes could be counted easily with the use of either diluent; in these cases further observations were not made. If the count could not be

made by using 1 per cent acetic acid, trial counts were made each twenty-four hours by both methods. This was done in eight cases. With 1 per cent acetic acid as a diluent, the leukocytes could not usually be counted until three to five days had passed, because of the unhemolyzed erythrocytes. When the leukocytes could first be counted with this diluent, the field under the microscope still contained some unhemolyzed erythrocytes. When the diluting fluid was tenth-normal hydrochloric acid, the leukocytes could always be counted easily and satisfactorily. It was apparent grossly in the pipette that the erythrocytes were hemolyzed. On the counting slide, the leukocytes were clearly visible and erythrocytes were not seen (table 1).

It is probable that diluents other than tenth-normal hydrochloric acid might be found as useful, but I did not attempt to test other fluids. The data presented do not afford a positive explanation for the failure of 1 per cent acetic acid to hemolyze the erythrocytes of patients who had been given infusions of solution of acacia. Perhaps the greater concentration of hydrogen ions of tenth-normal hydrochloric acid (pH 1.09) as compared to 1 per cent acetic acid (pH 2.80) is a factor. Nor are these data adequate for correlating the dose of acacia, in terms of grams of acacia for each kilogram of body weight, with the length of time that the leukocytes could not be counted, using 1 per cent acetic acid. Many of the patients were critically ill during the days the leukocyte counts were made, so that the increase in some cases cannot be considered as arising from injection of solution of acacia.

SUMMARY

After infusion of solution of acacia, it is often impossible to count the leukocytes in the usual manner, because the erythrocytes are not hemolyzed by the diluent (1 per cent acetic acid). By using tenth-normal hydrochloric acid as a diluent, however, the erythrocytes are completely hemolyzed and the leukocytes can be counted accurately.

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AN INEXPENSIVE OCULAR RULER TO FACILITATE RETICULOCYTE COUNTING

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The recognition of the importance of determining the "rate" of blood production has brought a general demand upon the present day intern and student body for numerous reticulocyte counts that may be very time-consuming without the proper microscope equipment.
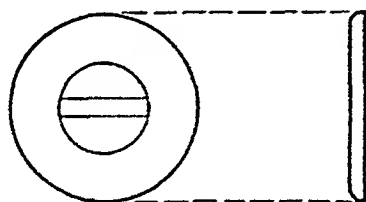

FIG. 1. CROWN GLASS DISC OF 21 MM. DIAMETER WITH TWO PARALLEL LINES 2 MM. APART

One of the chief difficulties has been the serial examination and enumeration of the stained cells in fields of the microscope containing a large number of corpuscles. Many have hesitated to request students to purchase high priced ocular micrometer discs or the still more expensive Ehrlich oculars which greatly facilitate these counts. Improvised markings on the lower lens of the ocular do not give clear-cut images and are difficult to clean off without damaging the lens.

To meet this demand I have designed a crown glass disc that fits rather loosely within the ocular casing and is supported on the ocular diaphragm. Across the center of the disc are engraved two parallel lines 2 mm. apart. In use with a thin film of corpuscles mounted under a cover glass in a mixture of brilliant

cresyl blue and sodium oxalate and viewed with the oil immersion lens a line of corpuscles is presented that may be readily counted in seriatim (see figs. 1 and 2).
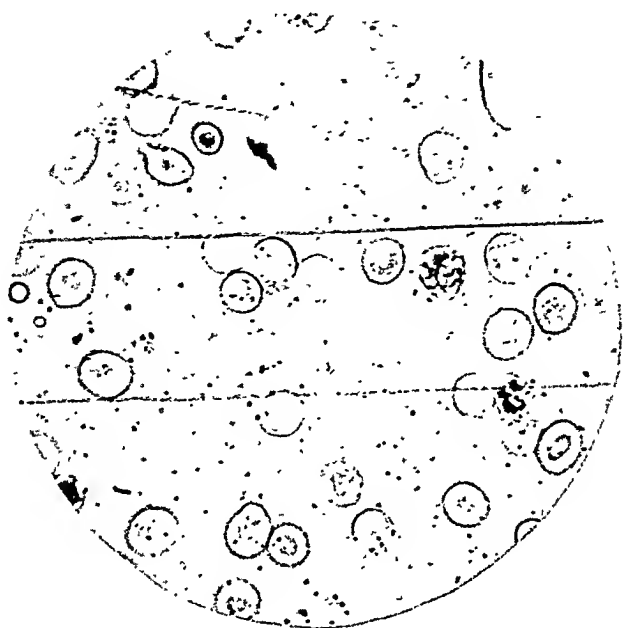

Fig. 2. The Central Portion of an Oil-immersion Field Showing Guide Lines, Erythrocytes and Reticulocytes in a Fresh Preparation Mounted Under a Cover Glass

This ocular ruler has been manufactured by the Bausch and Lomb Optical Company for our medical students at a cost of about a half dollar apiece.

EDITORIAL

The Problem of Trench Mouth

Literature, especially on dentistry, abounds in papers under the headings of Vincent's disease, stomatitis, angina, trench mouth, Gilman's disease, Plaut's disease, gingivitis, ulcero-membranous stomatitis sounding like the variegated, ancient terminology of Bright's disease. Some of the thousand and one indefinite abstracts of recent years have repeatedly reiterated and popularized an acute ulcero-membranous mouth lesion due to the usually described combination, Vincent's spirillum and fusiform bacillus. Because these bacteria are considered as anaerobes, the lesions are locally treated by so-called oxygen-excess producing perborate of sodium and peroxide of hydrogen, often needless and useless intravenous arsphenamine and such escharotics as chromic and trichloracetic acids.

Recently, *Time* tersely reported the subject based on facts and fallacies of the literature in an able, popularly written review sounding in the end like a possibly inspired advertisement for the use of an expensive rather than a cheap preparation of inadequate perborate of sodium. Incidently *Time*, misinformed, advocated the intramuscular use of neosalvarsan.

The prevalent Vincent's disease or trench mouth is an acute, ulcerative, necrotic, membranous, edematous, hemorrhagic condition usually primarily involving the gingivae, less often the tonsils. The tongue, floor of the mouth, fauces, lips, sides of the cheeks, often secondarily associated are rarely primary, and when so affected, without typical gingival reaction, are due to some other specific factor.

The easily recognizable, freshly smeared spirilliform organisms, *Borrelia vincenti*, are always the long, extremely active type, in these acute cases. Their presence alone is sufficient for a diagnosis. The frequently associated fusiform bacillus, *B. fusiformis*

(Hoelling), may be present in small numbers, may play a minor rôle, may be almost absent or may appear actively motile in large numbers. The characteristic clinical odor is not produced by these organisms but by other types easily cultured on blood agar plates. Still other associated cultural organisms, streptococcus, stapyhlococcus and short bacillary forms, are often of prime importance, although usually not considered.

The disease is now reportable in several States and it has a world wide distribution. If vacationists have imported many cases from Europe, they have exported just as many and the balance of trade is equal. The greatest age incidence is young adult life, equally prevalent in boys and girls; my youngest case age six was a school infection probably a pencil borne fomes, the older cases usually follow dental extractions. Direct infection by kissing is responsible for most of the cases but fomites are always possible. Public drinking fountains where the mouth touches any part are very bad; the common communion cup is obsolete. One attack does not immunize in the strict sense nor does it increase susceptibility. The greatest danger, aside from the cases of immediate death, lies in the insidious progression to chronic suppuration with the eventual development of so-called pyorrhoea, loss of teeth and focal infection.

The disease reaches its fastigium in from five to ten days, the mouth is always very sore, eating is painful. By ceasing to brush their teeth because of pain and bleeding, the latter always very profuse, patients increase the cultural pabulum. The gingivae are intensely reddened, very boggy, bleed at the slightest touch, the whole or portions being involved. The exudate is thin and sticky, teeming with active mixed bacterial types, *Borrelia vincenti* predominating. General reactions vary from slight to high temperatures and prostration. A leukocytosis prevails but leukopenia may be found. Agranulocytosis is frequent enough to warrant taking a blood count on every case. Death as a sequela may result if after dental extractions ensuing spirilliform gingivitis and cellulitis of the neck are not controlled.

The recognition of this type of gingivitis is a responsibility of the medical man; the exact bacteriological diagnosis belongs to the laboratory, and the treatment to the dentist.

Intelligent, specific chemotherapy directed at the local condition of the mouth with general supportive hospitalization regime will easily control the situation in twenty-four hours, will have the case well in hand on the third to the fourth day, and will eliminate all evidences or traces of the disease in from ten days to two weeks. The earlier the recognition, the more prompt are the results; if the severe cases of cellulitis in the neck are not too far advanced while they will respond, they of course do so more slowly. Intelligent treatment does not include chromic acid, trichloracetic acid, perborate of sodium and arsphenamine intravenously, all of which are now commonly used. It does include arsphenamine locally, as well as the dyes of which acriviolet is a very valuable remedy in spite of a recent communication to the contrary in the *Journal of the American Medical Association*, the more recent commercial mercurials, the silver salts, Dakin's solution, at times peroxide of hydrogen and pure castile soap. The second most important factor in treatment is the method and frequency of application. Under such a regime of recognition, study and treatment acute ulcerative spirilliform gingivitis, trench mouth or Vincent's disease, is a disease in which the results are most satisfactory both to patient and physician.

ROBERT A. KEILTY.

NEWS AND NOTICES

Although each meeting of the Society seems better than the preceding one, it will be difficult for a subsequent meeting to surpass the success of the Eleventh Convention held in New Orleans from May 6 to 9.

The local committee, with Dr. F. M. Johns as chairman, left no stone unturned to have every detail worked out to furnish the maximum pleasure and profit to the attending members.

The outstanding single feature was an all day trip to the Leprosarium, where the Society was the guest of Major O. E. Denney. After a delightful dinner in the old mansion house, clinics were held and laboratory demonstrations made.

Another interesting feature was a complementary supper given by the Hotel Jung after which a roundtable discussion extended well into the night.

On Monday, May 9, the Society dedicated the new Department of Pathology of the Louisiana State University, at which time Dr. H. J. Corper and Dr. T. B. Magath made the addresses and Dr. Walter Simpson and Dr. A. G. Foord performed autopsies. Dr. F. M. Johns unveiled the dedicatory tablet.

The scientific part of the program was unusually good, especially a morning program devoted to hematology. Free and valuable discussion followed the reading of the papers.

The complete account of the business meeting will be given in a later issue of the JOURNAL.

The Ward Burdick Medal was awarded to Dr. B. S. Kline for his work on tests in syphilis and the exhibit award was made to Dr. R. R. Kracke for his exhibit on agranulocytosis.

The Scientific Exhibits included the following:

(1) DR. R. D'AUNOY AND STAFF: Pathological specimens of unusual interest and x-ray visualization of the spleen and liver following administration of thorium dioxide.
(2) DR. ROY R. KRACKE: Blood and marrow studies of agranulocytosis.

(3) DR. B. S. KLINE: The "ball" test for syphilis.

(4) DR. F. M. JOHNS: (a) A pump for transfusion and other purposes utilizing the elastic recoil of the rubber tube to produce a vacuum and simultaneously forcing an almost continual flow of fluid through a system of tubes; (b) A reflex microscope for study of lesions of the crystalline lens; (c) The Johns precipitin test for syphilis, utilizing the increase in precipitating action of dried serum in the place of inactivated serum, also a combined drying and reading rack for use with this test.

The following were elected to Honorary Membership:

COL. C. F. CRAIG, United States   DR. E. C. DODDS, England
          DR. E. C. ACHARD, France

The following were elected to membership:

CURTIS, STEPHEN HORACE   GRILL, JNO. C.
BANKS, HORACE McMURRAN   BARTA, E. F.
FRESHMAN, WM. ALEXANDER   BOHLS, SIDNEY WM.
LINDBERG, LUDWIG A.   STOVAL, WM. DAVIDSON
JAISOHN, PHILIP   HULSEY, SIMEON HARDIN
HADEN, RUSSELL LANDRAM   POTTENGER, JOSEPH ELBERT
THORNTON, H. C.   MANER, G. D.
          DARDINSKI, V.

The following were elected to associate membership:

YAGLE, MARGUERITE E.   SCHERAGO, MORRIS

The following officers were elected:

President-Elect: DR. A. G. FOORD
Vice-President: DR. F. M. JOHNS
Members of the Executive Committee: DR. H. J. CORPER
          DR. C. I. OWENS
Members of the Board of Censors: DR. F. H. LAMB
          DR. W. S. THOMAS
Members of the Board of Registry: DR. PHILIP HILLKOWITZ
          DR. KANO IKEDA

BOOK REVIEWS

Microscopic Slide Precipitation Tests for the Diagnosis and Exclusion of Syphilis. By B. S. KLINE. Pp. viii + 99, 1932, Baltimore, The Williams & Wilkins Company, $2.50.

In the last few years there has been a tremendous development in precipitation and flocculation tests for syphilis. Dr. Kline has been one of the foremost contributors to this subject and in the present volume brings together the results of his researches up to the present time. While this book is primarily intended for the individuals who are going to actually perform these tests, it will prove valuable to the physician who is interested in syphilis, since it contains important information relative to the evaluation of such laboratory procedures According to the author the slide precipitation test differs from flocculation tests in general in that (1) the antigen is the acetone insoluble fraction only of alcoholic heart extract, (2) the antigen is more stable and more uniformly sensitive than those in general use, (3) the reactions are carried out on optimal open polished surfaces of microscopic slides in cells of optimal proportions and the results are read accurately with the aid of a microscope.

The book gives in detail, and with many illustrations, the exact method of performing the Kline test; both the diagnostic and exclusion slide tests with heated and unheated serums and with spinal fluids. A section of the book is devoted to a clinical consideration of the test and the author indicates that this test is 10 per cent more sensitive than the Wassermann reaction.

A preliminary report is made of the "ball" test which is a tube test using the centrifuge to cause the development of the ball. There is an excellent bibliography at the close of the volume. This small book will find great use in the laboratory since it brings together in a few pages the things necessary to know about these tests.

Biochemistry in Internal Medicine. By MAX TRUMPER AND ABRAHAM CANTAROW. Pp. 454, 1932, Philadelphia, W. B. Saunders Company, $5.50.

This book is primarily intended for clinicians and is an attempt to bring together the modern advances in physiology and biochemistry as encountered daily in internal medicine. The authors have made no attempt to make a laboratory manual out of the volume and give only a few technical methods. While the authors refer to various contributors to this field, only nine references are given in the text which will make the book of little value to the critical student. The book will give to the clinician a general insight into the application of some of the new discoveries in physiology and chemistry as applied to every day medicine. Much of the material contained in this book can be found in general laboratory manuals but the authors have, by leaving out the technical details, made the material more readable. The book will prove of most value to the clinician who does not have the time or the training to acquaint himself with the details of scientific advances in medicine, but does have time to read a short summary of the outstanding contributions to physiology and chemistry as related to specific diseases.

PROSPECT AND RETROSPECT*

H. J. CORPER

National Jewish Hospital, Denver, Colorado

In filling this most signal position this evening, I have only one regret and that is that this pleasure could not have been enjoyed by my colleague and friend who sacrificed so much in time and effort to establish this Society for a cause he sincerely believed in. During the infancy of this Society the rest of us proved only counselors and team mates and I am certain I voice the opinion of all who were fortunate enough to have played any part in that august occasion, the birth of The American Society of Clinical Pathologists, almost a decade ago, in the Missouri Baptist Hospital on May 22, 1922, at 8:00 p.m. I hope we shall never cease to pay tribute to the sacrifices of Ward Burdick for the welfare of the practicing clinical pathologist.

Dr. Burdick frequently reminisced about that first meeting attended by about forty men and a few ladies; very few of those present were personally acquainted with one another when Dr. George Ives of St. Louis called the assemblage to order for the "first successful national meeting of clinical pathologists," but the parting at the close of the meeting found East and West, North and South as friends united in one worthy effort—a ship embarked on an important voyage with able officers and committees in charge. It is hardly fitting to review names as they are all well known to you and I might be accused of the error of willful omission which, I assure you, should make me regretful. However, in retrospect I choose this course, risking possible criticism, to refresh your memory historically speaking and to satisfy a personal pride in the accomplishments of my friends and colleagues.

There were many hours of arduous labor on the part of the founders such as our first president Dr. Philip Hillkowitz and the early members. Colorado and Texas were preeminent in the early history because they had existing state societies of clinical pathologists which acted as the impetus for the national organization. It was Dr. Burdick's hope that some day every state would have a society like that in Colorado.

Dr. William Carpenter MacCarty of Rochester, Minnesota, proposed the appointment of a committee of five to carry out the purpose of that first day of the convention in St. Louis the founding of the society, and to submit a Constitution and By-Laws for consideration. The next morning saw such a Constitution prepared and an extemporaneous scientific session from the talent present was ready for the afternoon gathering. The number of charter members at the close of the first annual convention was 145.

The Second Annual Convention, June 25–26, 1923, was held in conjunction with the San Francisco meeting of The American Medical Association and in spite of the distance, more than seventy members registered.

About this time the organization accepted The Journal of Laboratory and Clinical Medicine as the official organ for its transactions which prior to this had been sent out in pamphlet form. Dr. MacCarty pointed out that the chief object of the Society was active coöperation or for the interest and mutual benefit of its members. He stated that the formation of the Society was stimulated by: (1) The necessity of picking out the practical diagnostic and therapeutic laboratory facts and methods from those of pure scientific interest; (2) the necessity of standardization of these facts and methods; (3) the necessity of setting a standard educational requirement for laboratory consultants to obviate the rapid increase of false claims of technicians who frequently conduct commercial laboratories, the value of which many physicians are unable to determine; (4) the necessity of maintaining laboratory sciences within the realm of the profession of medicine and not allowing them to become merely commercial adjuncts; (5) the stimulation of the interest of the

general profession in the real value of the laboratory in the practice of medicine and surgery; (6) needed assistance in the standardization of hospitals.

The Third Annual Convention was held in Rochester, Minnesota, June 6 and 7, 1924. The members were treated to the hospitality of The Mayo Clinic and its members, and at this meeting business matters and economic topics were relegated more or less to the background while science held sway. Round Table discussions were initiated to the delight and illumination of all present. At this time Dr. William J. Mayo and Dr. Malcolm T. McEachern paid tribute to the clinical pathologist's efforts. More than fifty applicants for membership were voted on favorably at the business session of the Convention. A symposium was held on the standardization of laboratory procedures.

In the transactions of the meeting under "News and Notes," we find that

"The American Society of Clinical Pathologists, however, is a young, vigorous and actively growing organization with strong proliferative tendencies aiming to keep the members constantly in touch with one another and spurring them on to better scientific and technical perfection of their specialty. The clinical pathologist is in the best strategic position to promote the success of the hospital standardization program. The American College of Surgeons recognizing the important rôle played by the hospital pathologist has been in close touch with and has actively coöperated with the American Society of Clinical Pathologists in their common endeavor to raise the medical level of the hospitals of this continent. At the section meetings arranged by the college in various parts of the United States a clinical pathologist has been placed on the program to discuss the problem of the laboratory."

Then follow notes on the competent supervision of hospital laboratories and a survey of hospital laboratories.

The Fourth Annual Convention was held in the ball room of the Benjamin Franklin Hotel at Philadelphia on May 20–23, 1925, with Dr. John A. Kolmer presiding. A feature of this meeting was a report on laboratory standardization which was discussed by Dr. Ruth Gilbert of New York and Dr. M. P. Colwell of The American Medical Association.

A pertinent suggestion at this meeting was a report from the Committee on Publication that the Society was considering pub-

lishing its own journal but that this was not deemed advisable for the present, and we heard for the first time that the Society had established a Service Bureau. The matter of registration of the technicians was considered of sufficient importance to be referred to the Executive Committee. At this time public health, state, city and county laboratories also came up for discussion and Dr. Sondern, upon accepting the presidency, pledged himself to solve the problem "as best we can."

A special feature of the Philadelphia meeting was the initiation of a commercial and scientific exhibit which proved such an outstanding success, both for the members and exhibitors, that it was considered advisable to continue this.

Notable also was the valid objection to considering "a clinical pathologist as a manipulator of fixtures and inanimate substances." As outlined by Dr. Herman Spitz, "a clinical pathologist must be a physician, primarily and basically," and "clinical pathology is the science of interpreting morbid processes as determined by means of various laboratory aids and correlating them with clinical symptoms."

With the news that the Fifth Annual Convention, in 1926, was to be held in Dallas, Texas, on April 15–17, there was great rejoicing because from Texas, as one of the pioneer states, originated the idea of the national society. The names of Drs. Moursund and Black will always remind those present of a great time.

With this came the valuable suggestion of the Past-President, Dr. John A. Kolmer, for an official text on "Approved Laboratory Methods in Clinical Pathology." It is rather singular that a Research Committee should have been appointed at this time by the President to investigate the question, and study the advisability of publishing the book of approved methods suggested by Dr. Kolmer.

The awarding of a prize to stimulate original research and an official publication of the Society were discussed at this time.

Illness prevented the Secretary-Treasurer, Dr. Ward Burdick, from attending the meeting but his energetic efforts were evident in the smooth conduct of the affairs.

The Sixth Annual Convention held in Washington, D. C., May

13–16, 1927, saw close coöperation with the American College of Surgeons in raising to the highest level all laboratories and standardized hospitals. The laity had become educated to the important rôle the pathologist plays in the team work of the hospital. Here for the first time we heard a report from the Committees on Research and a report by Dr. Kano Ikeda on the Registration of Technicians, features of the Society which have now assumed important proportions.

The meeting convened under Dr. William G. Exton and notable among the guest speakers were Dr. George K. Burgess, Director of the Bureau of Standards, Dr. George W. McCoy, Director of the U. S. Hygienic Laboratories, Washington, D. C. and Rear-Admiral E. R. Stitt.

Following the report of the Publication Committee by Dr. Kolmer, it was suggested that the Committee appoint the editors and associate editors and that work be begun at once on the new book on clinical pathology.

The Seventh Annual Convention greeted the members in Minneapolis, June 8–11, 1928 with Dr. A. H. Sanford in the Chair. The Society mourned the untimely demise of the Secretary, Dr. Ward Burdick, but his inspiration made all feel that he was still carrying on. Companionship was the keynote of this meeting under the excellent arrangements of the local committee headed by Dr. Charles R. Drake. The scientific and commercial exhibits proved a valuable asset due to the untiring efforts of Dr. A. C. Broders and Dr. Kano Ikeda.

To pave the way for possible future expansion and publication of a Society journal, the dues at this time were increased to a maximum of $10.00. Also a notable feature of this meeting, aside from the excellent scientific program, was the paper by Dr. William O'Brien on "The Cults." Another important feature of this meeting was a discussion on associate membership and their ethics. In addition an endorsement of the efforts of approving of laboratories by the American Medical Association was favored.

The Research Committee recommended the establishing of a yearly "Ward Burdick Research Award."

During this meeting also a most laudable innovation was initi-

ated by Drs. Charles Sheard and A. H. Sanford "for turning over to the American Society of Clinical Pathologists the privilege of exercising control over the commercial production of their new instrument for hemoglobin determinations."

At this time Dr. A. H. Schade suggested a revision of the application blank for membership which was subsequently executed.

In the February, 1929 number of The Journal of Laboratory and Clinical Medicine we note a proposed working scheme of the Registry of Technicians including the objects, the Board of Registry, the classification of laboratory technicians, the registration of schools for laboratory technicians, a placement bureau, and a code of ethics, and are advised that this Registry is now functioning quite satisfactorily and that technicians are rapidly availing themselves of the opportunity to obtain certificates.

In the March, 1929 number we are advised of the establishment of a "Ward Burdick Research Award" of the American Society of Clinical Pathologists dedicated to a man who was never too busy to answer a call on behalf of the Society. The award consisted of an appropriate gold medal bearing the profile likeness of Dr. Ward Burdick, and the nature of the award on its face, and on the reverse side the seal of the Society, a laurel wreath, and a place for the name of the recipient and the date of presentation.

The Eighth Annual Convention of the American Society of Clinical Pathologists was held in Portland, Oregon, July 5–8, 1929 with Dr. F. W. Hartman in the Chair. Drs. H. H. Foskett and C. H. Manlove will always be remembered for their excellent arrangements and western hospitality. It had been the custom throughout these years to hold the meetings and banquets in the headquarters hotels. This offered the unusual opportunity for fraternizing which always proved delightful and carried fond remembrances of friendship.

At this meeting for the first time the "Ward Burdick Medal" was presented to Dr. Walter M. Simpson of Dayton, Ohio for investigations in tularemia.

In 1929, we note the custom of official representation with other societies which was well exemplified in the presence of five representatives at the 19th Clinical Congress of the American College of Surgeons at Chicago, October 14–18, 1929, and two representa-

tives at the 58th Annual Convention of the American Public Health Association in Minneapolis, September 30 to October 5, 1929.

At the Portland meeting we learned that the text on Clinical Pathology was progressing rapidly and that the publication of a Journal by the Society was being further pursued.

The Committee on Exhibits by Dr. C. H. Manlove had presented eight exhibits, a laudable showing, and at this meeting the Board of Registry was officially acknowledged in the Constitution.

In 1930, we were advised that the scientific exhibits had assumed such proportions under the able supervision of Dr. C. I. Owen of Detroit, that two prizes, a gold and a silver medal were to be awarded.

The Ninth Annual Convention was held in Detroit on June 20–23, 1930, under the able leadership of Dr. J. H. Black of Dallas, Texas. A special feature of this meeting was a visit to Parke-Davis and Company under the kindly guidance of one of our Fellows, Dr. Walter E..King.

At this meeting the names of Dr. William H. Welch, Rear-Admiral E. R. Stitt, and Dr. Louis B. Wilson were proposed for honorary membership.

The Publication Committee reported that the book on Clinical Pathology would be ready for publication next year. The first awards for scientific exhibits were made, the gold medal being presented to Dr. E. R. Mugrage and Dr. Rodney H. Jones, and the second award to Dr. T. J. Curphey.

The Executive Committee at a post-meeting session June 23, 1930, authorized Dr. John A. Kolmer to negotiate with The Williams & Wilkins Company for the publication of the Society's journal and the first number appeared in January, 1931, under the editorship of Dr. T. B. Magath and an associate editorial staff of Fellows of the Society.

An interesting side-light of the activities of the Society is the discussion of the subject of the training of lay technicians by Dr. Walter E. King before the Congress of Medical Education, Medical Licensure and Hospitals, in February, 1930, fulfilling an important function of the Society. Dr. King pointed out the importance of specifying the minimum of work for students and of classifying technicians.

The Tenth Annual Convention was held in Philadelphia, June 7–9, 1931, under the Chairmanship of Dr. Kenneth M. Lynch. One of the interesting features of this meeting was the privilege to visit the little old brick building in which Osler performed his autopsies and to see his autopsy table and examine his original protocols.   At this meeting the Editor advised the Society that a contract had been entered into with The Williams & Wilkins Company for the publication of the AMERICAN JOURNAL OF CLINICAL PATHOLOGY, and that three issues were off the press, and that the JOURNAL was to appear at bimonthly intervals.

As was noticed earlier, the success of the Society was assured as the result of the magnetic personality of its founders and leaders, and the untiring and unceasing effort on their part.   At the close of the First Annual Convention in 1922 the number of charter members totaled 145.   In 1926, the roster showed a membership of 350, while the 1928–1929 roster showed a membership of 380. At the Philadelphia meeting, in 1931, the Secretary reported 376 active members of the Society: a steady increase showing healthy growth of the Society from 1922 to 1930 in spite of the loss of members each year by deaths and by discontinuing practice. I am now advised by Dr. Giordano that our total membership for this year is 358, a decided drop from last year, and of these sixty-seven are in arrears for one year, eighteen for two years and thirteen have been dropped for non-payment of dues and five have resigned.

In retrospect we can hardly fail to feel that the Society has made remarkable progress in welding the friendships of the clinical pathologists, and that through the efforts of the Society the status of the clinical pathologist has been decidedly elevated in the eyes of his medical colleagues and in the minds of his lay friends including hospital executives and executive boards.   It would, however, be unfair to expect that the work is completed and that the problems facing the clinical pathologists are insignificant.   If ever there was a need for the Society such need becomes even more pertinent today.   A work well initiated that must continue and be elaborated upon.   It is with this thought that I chose to close my address to you, my colleagues, in prospect.

With your kind permission, I should like to prospect with a view to benefiting not only ourselves but also our friends the patients and colleagues in allied fields. To do this efficiently I feel that we should be willing to do honor to Fellows in our own midst who merit such honors for industry and accomplishment, and in addition we should not hesitate to pay warranted honor to our scientific friends in other branches of endeavors. And to this end we have invited guests to join us at our annual meetings and we have seen fit to present to our own Fellows appropriate awards, one dedicated to perpetuating the name of one who insured the early success of the Society by personal sacrifice and the instillation of ideals. During this year, your President, inspired by the request of some of our best Fellows took upon himself the responsibility of appointing a number of new committees, one of them being for the purpose of doing honor to some international figure who has contributed to the welfare of clinical pathology, and the Society by card ballot approved the unanimous recommendation of the committee by a 4 to 1 majority. It is hoped by the Award Committee and concurred in by your President, that this may be perpetuated by the Society until the problems of clinical pathology have disappeared. In line with the general policy previously executed by the Society, it is hoped that succeeding presidents will see fit to perpetuate some of the committees appointed this year by your President to assist in sponsoring friendships with other scientific groups. Needless to say, some of them may cease to function and will become automatically obsolete and should then be discontinued. If I may plead for my successors in this Chair, may I suggest that they be given every opportunity to appoint such new committees as they may consider of value to them during their period of office. Such power is automatically given the president in other distinguished societies and should be our generous attitude toward our presidents. It has become a pleasure to other societies to seek our coöperation and friendship and may I suggest that we encourage this by appearing on their programs and by presenting exhibits and demonstrations as representatives of the American Society of Clinical Pathologists.

It is with pleasure that I, as a Fellow of this Society, look for-

ward to an International Union of Clinical Pathologists. You will recollect we made the Secretary of the British Association a Corresponding Member of our Society, 1929, and in 1930 we initiated an Honorary Membership. Needless to say, that the aforesaid functions are represented by committees of our Society which I hope my successor will continue—The Research Committee, The International Award Committee, The Committee for Honorary Membership, The Committee on Necropsies, and The Committee on Exhibits to Hospital and Medical Associations. Permit me also to express my appreciation of the excellent work of our Registry of Technicians.

I cannot dwell long upon my hopes for the Society as such in prospect but feel that an expression of opinion based on my personal experiences and my hopes for the Society may prove of some value to the membership. If we are to abide strictly by our Constitution and By-Laws. I believe they need careful consideration and revision by a group of qualified Fellows and I should advise leniency but thoughtful wording in such revision. I believe the Society has reached a point where the offices of Secretary and Treasurer should be separated and that small annual expenditures should be within the discretion of these two officers while large expenditures exceeding say one hundred dollars for an individual purchase should be approved by the Society.

The Registry of Technicians is a separate department of the Society and should be treated as such and should not be under the Secretary.

And finally, after a complete and satisfactory revision of the Constitution and By-Laws, it might be well to have a Society Parliamentarian who without prejudices or biases can with fairness render parliamentary decisions when requested. In closing, I would request also that unanimous decisions when rendered by committees be given serious and favorable consideration by the Executive Committee when called upon for their decision.

I look forward to another decade of accomplishment and to a bigger and better American Society of Clinical Pathologists, actively participating in the functions of an International Association of Clinical Pathologists.

THE DEMONSTRATION OF MYCOBACTERIUM TUBERCULOSIS IN EXUDATES, TISSUES, AND BODY FLUIDS: CONCERNING GUINEA-PIG INOCULATION AND CULTURAL METHOD FOR THE DEMONSTRATION OF MYCOBACTERIUM TUBERCULOSIS

W. W. HERRMANN, G. H. HANSMANN, AND THELMA DeCAPITO

(*From the Departments of Pathology and Bacteriology, and Hygiene and Preventive Medicine of the State University of Iowa, Iowa City*)

Because of the protean features of the lesions produced by, and the widespread distribution of, *Mycobacterium tuberculosis*, the physician is frequently called upon to make examinations for the establishment of the diagnosis of tuberculosis. No greater service can be offered a patient and those closely associated with him than an accurate diagnosis of tuberculosis. No greater injustice can be done to an individual than erroneously to brand him as tuberculous when such a false diagnosis could have been obviated. It is apparent that when a diagnosis of tuberculosis is under consideration methods of examination are still inaccurate. Any method which may increase accuracy in diagnosing tuberculosis should be given thorough and sympathetic attention.

The safest ground upon which a diagnosis of tuberculosis may rest is the recovery and identification of the etiological agent from the suspected lesion. One of the simplest methods, and hence the one most widely used, is the examination of a smear from the lesion stained immediately by one of the acid-fast methods. Pottenger demonstrated that this method is inadequate in many cases, since upwards of 100,000 organisms must be present per cubic centimeter of material in order that they may be consistently found in smears.

The laboratory worker is always on the alert for a more delicate method with which he may improve his armamentarium for the demonstration of *Mycobacterium tuberculosis*. According to

Feldman and Magath,[7] as early as 1867, even before the discovery of the organism by Koch, Marcet, advocated the use of guinea-pigs for the purpose of demonstrating the tuberculous nature of material from suspected cases of tuberculosis in man. This animal is highly susceptible to infection with the *Mycobacterium tuberculosis*, fifty organisms, according to Webb, Gilbert, and Havens,[13] being sufficient to set up systemic lesions of the disease. In addition, the guinea-pig is able to overcome a moderate number of contaminating organisms which may be inadvertently present in the inoculum. Guinea-pigs are readily available, have been demonstrated by Magath and Feldman to be the basis for a most reliable method of demonstrating the presence of *Mycobacterium tuberculosis* when present in small numbers, and therefore inoculation of guinea-pigs is the test most widely used in laboratories.

Among the objections to guinea-pig inoculation are the length of time (six to eight weeks) which must elapse before the typical lesions develop, the initial cost of the animals, and cost of maintenance during the time that lesions are developing, and a certain amount of danger of the animal's dying from intercurrent infection. The time elapsing before a positive report may be returned is shortened, it is true, if local lymph-nodes are found to be enlarged and are removed for examination without sacrificing the animal. This procedure entails considerable time, however, as compared with the examination of a tube of culture medium. Then, too, the questions although not very important as to whether some guinea-pigs may not be resistant to infection or, on the other hand, develop spontaneous tuberculosis, always enter into a discussion of the guinea-pig method.

The cultivation of *Mycobacterium tuberculosis* on artificial mediums followed almost immediately the discovery of the organism. Pawlowsky[10] pointed out the usefulness of potato medium soon after Koch's discovery. Dorset later showed that eggs contained the food requirements for the cultivation of *Mycobacterium tuberculosis* and later Petroff[11] added a bacteriostatic dye to Dorset's[5] medium in order to inhibit the growth of contaminants. Within the last few years many other formulas for the

preparation of mediums suitable for the growth of these organisms have been proposed.   It was not the purpose of this study to compare of the relative efficacy of these various mediums in cultivation of *Mycobacterium tuberculosis* from suspected material, but rather to select one such medium, which had shown promising results, and subject it to prolonged parallel study with guinea-pig inoculation which was in use in this laboratory, for the routine demonstration of the *Mycobacterium tuberculosis* in tissues, exudates, and body fluids.

The study was begun early in 1930 and an attempt was made, as far as practicable, to use consecutive materials as they were submitted to the hospital laboratory for examination with reference to the presence of *Mycobacterium tuberculosis*.   Where the material submitted was so meager that it appeared the guinea-pig test would be invalidated by dividing it, only the guinea pig was inoculated.   Because of a changing personnel in the bacteriological laboratory, otherwise available material was discarded without being cultured.   However, up to July 1, 1931, 527 parallel attempts had been made to recover the *Mycobacterium tuberculosis* by guinea-pig inoculation and by culture.

PROCEDURE

The culture medium selected was the crystal-violet, potato-slant preparation described by Corper and Uyei.   At first the inoculum was treated with 6 per cent sulphuric acid before seeding, but later, when the authors reported more favorable results with 5 per cent oxalic acid, the latter method of inhibiting the growth of contaminating organisms was used.   The cultures were made by one of us (T. D.), in the State Bacteriological Laboratory, three tubes being seeded with each specimen after being treated in the prescribed manner.   Fluids were centrifugalized before treatment and the solid specimens were triturated with a sterile mortar and pestle.   At first the seeding was done with a loop.   Later it was felt that a quantity of inoculum, more comparable to that received by the guinea-pig would be used, if the seeding were done with a sterile pipette which permitted the inoculation of several drops of the centrifugalized specimen, and that modification was used thereafter.   Cultures were observed weekly and, unless growth appeared, were under observation for a total of twelve weeks. The culture was considered to be positive if the characteristic, dry, yellow, granular, piled-up colony developed, which, when smeared out and stained, was proved to be composed of acid-fast, bacillary organisms.

The guinea-pigs were inoculated subcutaneously near the groin. Fluids were injected by means of a syringe and hypodermic needle, while solid material was inserted through a small skin incision which was then closed by suture. Usually only one guinea-pig was used for each specimen. These animals were then kept in small cages in the animal room, not more than four and usually two or three animals to a cage. All animals were sacrificed at the end of six weeks if they had not died before that time. Post-mortem examinations were made of all guinea-pigs as soon after death as possible. The usual evidences of tuberculosis are a local, dirty, weeping ulcer at the site of inoculation, enlarged, inflamed and sometimes caseous lymph-nodes draining the site of inoculation, and an

TABLE 1

COMPARISON OF GUINEA-PIG INOCULATIONS AND CULTURES

	TISSUE	URINE (URETERAL)	URINE (VOIDED)	URINE (BLADDER)	EXUDATE	SPINAL FLUID	SPUTUM	STOOL	GASTRIC CONTENT	CYST FLUID	TOTAL
Guinea-pig negative, culture negative.....	48	202	10	10	70	15	5	2		1	363
Guinea-pig positive, culture positive......	15	17	2		10	3			1		48
Guinea-pig positive, culture negative.....	9	4			11	3			1		28
Guinea-pig positive, culture incomplete...		2		1	1						4
Guinea-pig negative, culture positive.....	3	2	1		1						7
Guinea-pig incomplete, culture positive...	4				1						5
Guinea-pig negative, culture incomplete..	2	13		2	5	1					23
Guinea-pig incomplete, culture negative..	9	20		3	8		3				43
Guinea-pig incomplete, culture incomplete..................................	1	1	2		1					1	6
Total..............................	91	261	15	16	108	21	9	2	2	2	527

enlarged spleen which contains many small, white, discrete, somewhat firm tubercles. A guinea-pig was considered positive if it exhibited one or more of these gross findings from which typical acid-fast rods could be demonstrated by direct smear and stain.

COMMENT

Table 1 indicates the source and character of the various materials used and the results obtained. In 411 instances there was perfect agreement between the guinea-pig inoculation and culture, 363 negative tests and forty-eight positive tests. There were thirty-five instances of disagreement; twenty-eight instances in which the guinea-pig was positive but no growth

appeared in the culture tube, and seven instances in which there was a typical growth on the culture medium but no lesions could be demonstrated in the animals.   In six of these seven instances, the organism recovered by culture was injected into guinea-pigs and produced typical tuberculous lesions containing acid-fast bacilli.   In the other instance, the culture was no longer available for an animal pathogenicity experiment.   There was no clinical evidence of tuberculosis in the urinary tract of this patient, the specimen being ureteral urine obtained by catheter, and no further organisms were isolated on repeated attempts. In all, six guinea-pigs and six sets of cultures were made from catheterized ureteral urines of this patient.

Eighty-one of the 527 tests were incomplete in that either the culture tubes or guinea-pigs or both could not be properly evaluated.   The chief reason for failure with the guinea-pig test was death of the animal shortly after inoculation and before tuberculous lesions had time to develop.   Death was presumably due to infection with other organisms in the inoculum and occurred usually within seventy-two hours.   The causes of failure with the cultures were equally divided between contamination, loss by breakage in the centrifuge, and prolonged treatment with acid. The work was carried on in the midst of a heavy routine so that in a few instances the material was subjected to contact with acid for a longer period of time than that recommended by Corper and Uyei.[2,3]   It is doubtful, from the experience of Woolsey,[14] if this prolonged acid treatment had any effect on the *Mycobacterium tuberculosis*, but such cultures were classed as incomplete.

Thus, considering only the positive reports in which both tests were complete, the guinea-pig method surpasses without question, the culture method.   If, however, the negative findings and the incomplete tests are included, even though they may be of doubtful clinical value, the two methods more nearly balance, the guinea-pig tests being incomplete in fifty-four instances, whereas the culture results were incomplete in thirty-three.

Hohn[8] believes that the question is not so much which of the two methods, culture or animal inoculation, is the superior, but rather, which type of material should be cultured and which

inoculated into a guinea-pig.   We had this point in mind when
we began our comparison.   A study of the table fails to show any
particular type of material which gives decidedly better results
with one method than with the other.   There are instances in
each of the major brackets in which either of the methods was
positive in the face of a negative test by the other method.

Among the 363 instances in which both tests were completed
with a negative result, there were twenty-two clinical cases in
which the diagnosis of tuberculosis had been made with respect
to the lesion in question, in spite of the inability to demonstrate
the organism.   In two of these cases the diagnosis was subse-
quently substantiated by the demonstration of *Mycobacterium
tuberculosis.*

Hohn,[3] Corper,[1] and Corper and Uyei,[4] each with the method
developed in his own laboratory, report results equal or superior
to the guinea-pig inoculation in the recovery and the identifica-
tion of *Mycobacterium tuberculosis* from suspected materials.
Woolsey, using Herrold's medium, finds agreement in 93 per
cent of 130 cases and superiority of the medium over the animal
in five of the nine cases of disagreement.   Feldman,[6] Stadnich-
enko and Sweany,[12] and Lutz,[9] using various mediums including
Hohn's and Corper's, on the other hand, consider the animal in-
oculation to be superior to cultural methods.   This, too, is the
position that we take at this stage of the study.   We believe
that cultural methods have a definite place in the laboratory
diagnosis of tuberculosis and the demonstration of the organism.
As the table indicates, there are eleven instances, leaving out the
one doubtful case, in which *Mycobacterium tuberculosis* was dem-
onstrated to be present in material by means of the culture tube,
in which the guinea-pig either died before the disease developed
or else was negative when examined for tuberculosis at the end
of six weeks.   On the other hand there are so many instances in
which the guinea-pig method surpassed the cultural method,
that we believe it to be the superior method as a routine proce-
dure.   The series is being continued in an attempt to improve
our technique, hoping to approach the favorable results reported
by Corper and Uyei[2] and others.

CONCLUSIONS

1. Five hundred and twenty-seven parallel animal inoculations and cultures have been carried out to demonstrate the *Mycobacterium tuberculosis* in tissues, exudates, and body fluids.

2. Guinea-pig inoculation has surpassed the cultural method for the demonstration of *Mycobacterium tuberculosis* in our series.

3. Guinea-pig inoculation surpassed the cultural method for all types of inoculum.

4. The cultural tests showed the greater percentage of endeavors carried to completion.

5. Approximately 10 per cent of the patients who presented the clinical signs of tuberculosis and from whom the initial material from the suspected tuberculous lesion was negative for *Mycobacterium tuberculosis* by both methods, have, on subsequent employment of the same methods, been proved tuberculous.

6. The cultural method is a good supplementary method to use in conjunction with animal inoculation for the recovery of *Mycobacterium tuberculosis* from routine suspected materials.

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CHEMICAL AND BACTERIOLOGICAL STUDIES OF PYRIDIUM

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In the biochemical investigation of pyridium which we conducted, the first series of our bacteriologic tests were made to determine the antibacterial value in vitro of the product on *Escherichia coli* (B. coli), *Staphylococcus aureus*, Streptococcus (Gay), *Corynebacterium diphtheriae* and Pneumococcus.

Pyridium was chosen as representative of the azo dye group as it appeared to be chemically stable and definitely antibacterial in vitro. In a previously published report,[1] we showed that pyridium had definite bacteriostatic properties in concentrations of 1:4,000 to 1:10,000, the average being about 1:8,000, against *Esch. coli, Staphylococcus aureus, Corynebacterium diphtheriae,* Pneumococcus (Gay), and Streptococcus. The bacteriostatic properties were not inhibited to any marked degree by the addition of such biological fluids as urine and blood serum, when added to the mixtures of pyridium and organisms. The compound showed bactericidal properties for the test organisms in concentrations of 1:4,000 to 1:10,000 with an average of 1:5,000.

In view of these findings it was deemed advisable to test the bactericidal and bacteriostatic properties of pyridium in vivo, by investigating the urine excreted by individuals who were given varying doses of the dye. The report includes (a) measurements of the dye excreted by the evaporation and precipitation method, the colorimetric method and the concentration as based on a color index ratio, and (b) bacteriologic studies including control experiments, determination of the effect of pyridium on a series of urine specimens obtained from twenty-seven individuals, each taking 0.6 gram of pyridium per day and also on a series of urine specimens obtained from individuals taking 0.9

gram per day in three doses of 0.3 gram each, and on catheterized specimens from patients taking 0.6 gram per day. The specimens were inoculated with staphylococci and *Esch. coli.*

EXPERIMENTAL

Total dye excreted

The first series of experiments were intended to show the concentrations of dye excreted in the urine obtained from individuals who were taking six 0.1 gram tablets of pyridium daily. The tests were carried out over a period of seventy-two hours in the case of each individual. The total volume of urine for each day was measured and the dye content determined according to the following technique.

A. Evaporation and precipitation method. The total urine in each case was placed in large shallow porcelain dishes and evaporated to about one-tenth of the original volume by heating on an asbestos mat placed over an electric plate. The temperature was never raised above 30 to 35°C. In order to facilitate evaporation a current of air was drawn over the surface of the liquid. The rapid evaporation which resulted from this method also helped to keep the temperature low. The concentrated urine was then acidified with dilute hydrochloric acid and cooled for twenty-four hours by immersing the dish in cracked ice. The precipitate was collected on a weighed filter paper and washed with distilled water containing a few drops of hydrochloric acid and then washed with distilled water, dried in a desiccator and weighed.

Table 1 gives the total volume of urine (average, 994.5 cc.) excreted by each individual while taking 0.6 gram of pyridium per day, the total volume of liquid was restricted as much as possible in each case.

Table 2 records the daily excretion of pyridium as determined by the evaporation and precipitation method.

B. Total volume in seventy-two-hour period. Because of the rather low percentage of recovery of pyridium, a second series of experiments was conducted in which the total volume for a seventy-two-hour period was collected and evaporated according to the method used in the preceding series.

This method gives rather variable results apparently due to the fact that the pH of the precipitating mixture cannot be accu-

rately controlled. This is evident from the results recorded in table 2, where the percentages of recovery ranged from 12.7 to 56.1 with an average of 29.2. In the case of the second series where the urine was collected during a seventy-two-hour period the range of recovery of pyridium was from 32.47 to 44.05 per cent with an average of 36.25. The higher percentage of recovery in the second series is apparently due to the greater concentration of pyridium in the evaporated residue from a seventy-two-hour

TABLE 1

Average Daily Volumes of Urine Excreted in Ten Patients

FIRST DAY	SECOND DAY	THIRD DAY
1,001.5 cc.	1,059.5 cc.	921.5 cc.

TABLE 2

Average Daily Excretion of Pyridium in Ten Patients

FIRST DAY	SECOND DAY	THIRD DAY	PERCENTAGE RECOVERED
140.7 mgm.	181.35 mgm.	183.1 mgm.	29.2

TABLE 3

Average Excretions of Pyridium During a Seventy-two-Hour Period in Five Patients

VOLUME URINE	PYRIDIUM RECOVERY	PERCENTAGE RECOVERY
3,430	655 mgm.	36.25

sample of urine. This higher concentration permits a greater precipitation with hydrochloric acid. Because of this variation in percentage of recovery, and also owing to the low percentage of recovery, the colorimetric method was then tried.

C. The colorimetric method. The colorimetric method was found to give fairly satisfactory results. This method is carried out as follows:

Ten cubic centimeters of urine containing pyridium was made up to 500 cc. with distilled water in a volumetric flask. In this dilution the light color of normal urine is negligible. This can be demonstrated by taking 10 cc. of urine

and diluting to 500 cc. with distilled water when it will be found that the mixture cannot be differentiated in the colorimeter from distilled water.

The diluted pyridium in urine, which incidently has a marked color, is compared in a colorimeter with a standard solution made by dissolving 10 mgm. of pyridium in 500 cc. of distilled water.

The usual readings are made on the colorimeter and calculations made according to the following formula:

$$C_1 = \frac{R_2}{R_1} \times C_2 \times 50$$

C_1 represents concentration of unknown pyridium in urine. R_1 represents reading on the colorimeter of the unknown, while R_2 represents the reading of the standard solution on the colorimeter. C_2 represents the concentration of standard. The constant 50 represents the relation between the diluted and undiluted urine. By multiplying C_1 by 100 we can obtain the number of milligrams in 100 cc. of urine. For example, 10 cc. of pyridium urine diluted to 500 cc. showed on colorimetric estimation that 10 millimeters of standard was matched by 28.5 millimeters of diluted unknown. By substituting in our formula, we get the following:

$$C_1 = \frac{10}{28.5} \times 0.02 \times 50 = 0.35090 \text{ mgm. per cubic centimeter of undiluted urine}$$

In 100 cc. of undiluted urine there are 35.09 mgm. of pyridium.

Table 4 gives the daily volume of urine excreted over a three-day period in this experiment. There was an average of 977 cc. per day.

Table 5 shows the daily excretion of pyridium and the percentage of recovery as determined in the colorimeter.

The complete tabulation shows a percentage of recovery by the colorimetric method ranging from 67.1 to 80.5 with an average of 74.7. It should be noted that with the colorimeter more consistent results are obtained than with the evaporation-precipitation method. The former gives higher percentages of excretion of pyridium than the latter.

Inasmuch as the pyridium in the urine is not identical with pure pyridium ingested in the experiments above, numerous experiments were conducted in order to find a ratio between the color index of pyridium eliminant and that of pure pyridium. Solutions of each in pure distilled water cannot be matched up in the colorimeter because pyridium eliminant cannot be entirely

dissolved in water. Experiments were tried in precipitating the pyridium eliminant from the urine as a mercury compound after the phosphates, oxalates, and sulphates had been removed with barium chloride and checked against pure pyridium precipitated from solution with mercury salts. Our conclusions from these experiments only served to show that pyridium and pyridium eliminant were not the same chemically since they combined with different weights of mercury.

D. *Color index ratio.* Ten milligrams of pure pyridium was dissolved in 500 cc. of normal urine and 10 mgm. of pyridium eliminant was dissolved in another 500 cc. of normal urine using heat to aid solution of the compounds. This was easily accom-

TABLE 4

DAILY VOLUME OF URINE EXCRETED

FIRST DAY	SECOND DAY	THIRD DAY
875 cc.	1,195 cc.	861 cc.

TABLE 5

DAILY EXCRETION OF PYRIDIUM

FIRST DAY	SECOND DAY	THIRD DAY	PERCENTAGE RECOVERED
319 mgm.	428 mgm.	466 mgm.	74.7

plished. The solutions were made up accurately in volumetric flasks, cooled and final volume brought to 500 cc. The two solutions were then matched in a colorimeter and it was found on repeated trials that 30 mm. of the standard pure pyridium solution in urine was equivalent to 33.5 mm. of pyridium eliminant solution in the same urine. The ratio of color value of pure pyridium to pyridium eliminant is 0.895. Using this ratio in our calculations on the elimination of pyridium in the urine, we obtained for the seven subjects in table 5, an average of 65.7 per cent.

This colorimetric procedure gives far more constant results for elimination of pyridium in the urine and the figures are nearer

the truth with respect to the amount eliminated. This is seen by the fact that only at times do the results found by the evaporation-precipitation method approach the above. This method based on the colorimetric determination of other urine constituents is easy to carry out and like these basic methods gives better results than purely chemical methods for the estimation of substances in the urine, because of the great danger of side reactions between the reagents used and the many other compounds found in this complex excretion.

Bacteriologic studies on pyridium

Control study. As a preliminary control study the urine of ten individuals was collected twice daily, using the same precautions as described above. One cubic centimeter of urine was plated immediately with 10 cc. of nutrient agar, pH 7.2, incubated at

TABLE 6

Control on Normal Urine; Average Colonies in Ten Patients

FIRST DAY	SECOND DAY	THIRD DAY
119.7	87.2	40.9

37.5°C. for twenty-four hours and results recorded. During this trial the subjects received no pyridium, the test being conducted for three days (table 6).

In view of these findings it was not considered advisable to use too dilute a culture of test organisms in the actual trials with pyridium urine.

The bacteriological studies were conducted on twenty-seven normal individuals taking 0.6 gram pyridium a day for fourteen days. Urine was collected twice daily under aseptic conditions, as far as this was possible. Sterile containers were used to receive the urine. Only male subjects were used. The penis was thoroughly cleaned before urination and the first portion rejected. Ten cubic centimeter samples of urine were inoculated with 0.1 cc. of a 1:10 dilution of a twenty-four hour culture of the organism studied. Controls were run using 10 cc. of broth inoculating each with 0.1 cc. of a 1:10 dilution of organism. These always exhibited growth, showing viable organisms. Cultures were incubated at 37.5°C. for twenty-four hours and results recorded. The

control and the urine cultures were then plated, using 10 cc. of nutrient agar, pH 7.2, and 1 cc. of culture. The plates were incubated at 37.5° for twenty-four hours and results recorded. The original urine cultures were then incu-

TABLE 7

SUBJECT: G. S.   ORGANISM: STAPHYLOCOCCUS

DAY	TIME	CONTROL CULTURE IN BROTH	URINE CULTURES			COLONIES PER PLATE		
			24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
1	A.M.	+	—	—	+	0	2	u
1	P.M.	+	—	—	+	0	11	u
2	A.M.	+	—	—	—	0	0	0
2	P.M.	+	—	—	+	0	3	u
3	A.M.	+	—	—	—	0	0	0
3	P.M.	+	—	—	—	0	0	7
4	A.M.	+	—	—	—	0	0	0
4	P.M.	+	—	—	—	0	0	0
5	A.M.	+	—	—	—	0	0	0
5	P.M.	+	—	—	—	0	13	8
6	A.M.	+	—	—	—	0	0	0
6	P.M.	+	—	—	—	0	0	0
7	A.M.	+	—	—	—	0	0	0
7	P.M.	+	—	—	—	0	0	0
8	A.M.	+	—	+	+	50	u	u
8	P.M.	+	—	+	+	79	u	u
9	A.M.	+	—	+	+	0	u	u
9	P.M.	+	—	—	—	10	u	u
10	A.M.	+	—	—	+	14	103	u
10	P.M.	+	—	—	+	2	81	u
11	A.M.	+	—	+	+	7	u	u
11	P.M.	+	—	+	+	13	u	u
12	A.M.	+	—	+	+	0	73	u
12	P.M.	+	—	—	+	4	51	u
13	A.M.	+	—	+	+	0	u	u
13	P.M.	+	—	+	+	6	u	u
14	A.M.	+	+	+	+	179	u	u
14	P.M.	+	—	+	+	15	u	u

The twenty-four, forty-eight, and seventy-two-hour control plates were all uncountable.

+ = growth; — = no growth; u = uncountable.

bated at 37.5°C. for a third period of twenty-four hours, plated and results recorded. In this way the prolonged action of pyridium excreted in the urine could be studied in the case of each organism used.

The complete reports of Staphylococcus and *Esch. coli* bacteriologic studies

on the urines of individuals receiving pyridium were made after the plan of table 7, which constitutes a sample Staphylococcus protocol on one of the twenty-seven individuals. The data thus collected were recorded for each individual

TABLE 8

SUBJECT: J. J. McG. ORGANISM: ESCH. COLI

DAY	TIME	CONTROL CULTURE IN BROTH	URINE CULTURES			COLONIES PER PLATE		
			24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
1	A.M.	+	−	−	−	10	4	0
1	P.M.	+	−	−	−	18	6	14
2	A.M.	+	−	−	−	0	0	3
2	P.M.	+	−	−	−	0	14	1
3	A.M.	+	−	−	−	0	0	1
3	P.M.	+	−	−	−	0	4	6
4	A.M.	+	−	−	−	0	0	16
4	P.M.	+	−	−	−	1	1	1
5	A.M.	+	−	−	+	0	11	u
5	P.M.	+	−	−	+	7	2	u
6	A.M.	+	−	−	+	3	16	u
6	P.M.	+	−	−	−	6	0	17
7	A.M.	+	−	−	−	22	17	39
7	P.M.	+	−	.	+	34	6	u
8	A.M.	+	−	−	+	10	16	u
8	P.M.	+	−	+	+	17	40	u
9	A.M.	+	−	−	−	2	0	9
9	P.M.	+	−	−	−	4	11	5
10	A.M.	+	−	−	−	5	7	1
10	P.M.	+	−	−	−	1	4	0
11	A.M.	+	−	−	−	4	2	7
11	P.M.	+	−	−	−	22	11	16
12	A.M.	+	−	−	−	0	6	2
12	P.M.	+	−	−	−	0	4	12
13	A.M.	+	−	−	+	6	23	u
13	P.M.	+	−	−	+	6	9	u
14	A.M.	+	−	−	+	17	29	u
14	P.M.	+	−	−	+	4	18	u

The twenty-four, forty-eight, and seventy-two-hour control plates were all uncountable.

in the series of twenty-seven cases and for each of the test organisms used, namely, *Esch. coli* and *Staphylococcus aureus*. (See tables 7 and 8.)

Study of patients taking 0.3 gram pyridium three times daily. A test was made of the urine of several individuals who were

taking 0.3 gram of pyridium three times a day. The technique was the same as that followed in the case of the individuals taking 0.2 gram three times a day. It is apparent from the table that

TABLE 9

Subject: A. G.   Organism: Esch. coli

DAY	TIME	CONTROL CULTURE IN BROTH	URINE CULTURES			COLONIES PER PLATE		
			24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
1	A.M.	+	—	—	+	74	88	u
1	P.M.	+	—	—	+	84	56	u
2	A.M.	+	—	—	+	60	99	u
2	P.M.	+	—	—	+	19	65	u
3	A.M.	+	—	—	+	71	100—	u
3	P.M.	+	—	—	+	46	34	u
4	A.M.	+	—	—	+	88	38	u
4	P.M.	+	—	—	+	42	28	u
5	A.M.	+	—	—	+	105	45	u
5	P.M.	+	—	—	+	38	63	u
6	A.M.	+	—	—	+	13	121	u
6	P.M.	+	—	—	+	20	67	u
7	A.M.	+	—	—	+	22	80	u
7	P.M.	+	—	—	+	46	47	u
8	A.M.	+	—	—	+	111	49	u
8	P.M.	+	—	—	+	74	35	u
9	A.M.	+	—	—	+	13	66	u
9	P.M.	+	—	—	+	101	39	u
10	A.M.	+	—	—	+	68	84	u
10	P.M.	+	—	—	+	44	100	u
11	A.M.	+	—	—	+	56	46	u
11	P.M.	+	—	—	+	81	20	u
12	A.M.	+	—	—	+	70	68	u
12	P.M.	+	—	—	+	103	59	u
13	A.M.	+	—	—	+	44	47	u
13	P.M.	+	—	—	+	81	86	u
14	A.M.	+	—	—	+	54	77	u
14	P.M.	+	—	—	+	89	51	u

The twenty-four, forty-eight, and seventy-two-hour control plates were all uncountable.

there is no distinct advantage due to the increased dosage of pyridium (table 9).

Tests of catheterized urine. It was considered desirable to obtain catheterized specimens of urine from individuals taking

pyridium. The usual dose of 0.6 gram per day was given, and a daily catheterized specimen of urine was used for inoculation with *Esch. coli* and Staphylococcus. The tests were carried out for a period of seven days and one sample collected daily. In all other respects the experiments were carried out as in the previous work. Table 10 shows the results obtained with one individual.

TABLE 10

SUBJECT: F. L. H.　ORGANISM: STAPHYLOCOCCUS

DAY	CONTROL CULTURE IN BROTH	URINE CULTURES			COLONIES PER PLATE		
		24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
1	+	−	−	+	11	47	u
2	+	−	+	+	9	u	u
3	+	−	−	−	16	0	0
4	+	−	−	−	3	0	0
5	+	−	+	+	8	u	u
6	+	−	−	−	13	2	0
7	+	−	−	+	5	14	u

SUMMARY

The experiments dealing with the excretion of Pyridium in the urine as determined by the evaporation and precipitation method showed a range of recovery from 12.7 per cent to 56.1 per cent. This wide range might be explained by a difference in pH of the urine containing the pyridium eliminant. The colorimetric determinations were more constant; these showed a range of recovery from 67.1 per cent to 80.5 per cent, with an average of 74.7 per cent. The percentage of 65.8 based on the color index ratio was accepted as final.

The bacteriologic experiments showed a definite bacteriostatic action of pyridium against *Staphylococcus aureus* and *Esch. coli* when comparison was made between the test plates and the control plates. The bacteriostatic action was more constant than the bactericidal action. The bactericidal action showed a wide variance at times; the test plates showing no growth on some days. In the above experiments the dose of Pyridium was the usual one of 0.6 gram per day. Increasing the dosage to 0.9

gram per day did not appreciably affect the bacteriostatic and bactericidal findings.

Experiments in which the usual dose of pyridium was given and the urine samples obtained by catheterization showed bacteriostatic and bactericidal action against the test organisms, Staphylococcus and *Esch. coli.*

REFERENCE

(1) GOERNER, A., AND HALEY, F. L.: A study of the antibacterial properties of Pyridium, urinary antiseptic. Jour. Lab. and Clin. Med., **16:** 957–966. 1931.

AN EXPERIMENTAL STUDY OF THE ACTION OF PHENYLHYDRAZINE HYDROCHLORIDE AND ACETYLPHENYLHYDRAZINE (PYRODIN), WITH REFERENCE TO THEIR USE IN THE TREATMENT OF POLYCYTHEMIA VERA*

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Phenylhydrazine-hydrochloride ($C_6H_5 \cdot NH \cdot NH_2 \cdot HCl$) has become the most widely used therapeutic agent in polycythemia vera, despite its known effects as a powerful protoplasmic poison. Those who have considered the drug from the standpoint of its possible deleterious action in man have come to the general conclusion that in therapeutic doses, even though administered for an indefinite period, it is not particularly injurious. Admittedly, this compound has been used with comparative success, and perhaps for this reason most clinicians have overlooked the probable advantages of acetylphenylhydrazine, a substance equally effective in blood destruction, but which, certainly in acute intoxications, produces much less damage to the organism than phenylhydrazine. That the use of phenylhydrazine-hydrochloride involves a considerable element of danger has been noted by several clinicians, notably Giffin and Conner,[9] Gouwens,[10] and McNamara and Sansum.[14]

Stimulated by the researches of Fischer[7] on the reactions of phenylhydrazine with the aldehyde and ketone groups of carbohydrates, Hoppe-Seyler[12] investigated the effects of this compound in the animal organism. He determined that the action of the compound on the blood consisted in the formation of a characteristic, but hitherto unknown brown pigment, exhibiting sharply-

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defined absorption bands, which, however, changed readily into a substance that could not be distinguished by its absorption spectrum. The behavior of the free base, according to Hoppe-Seyler, differed from that of the hydrochloride in that, in the absence of oxygen, it converted the hemoglobin into hemochromogen.*

Following Hoppe-Seyler, experimental studies have dealt mainly with the effects of phenylhydrazine in producing experimental anemia, with alterations in the leukocyte picture and with the questions of hepatic and renal damage.

Interest in the toxicology of acetylphenylhydrazine, or pyrodin ($C_6H_5 \cdot NH \cdot NH \cdot CO \cdot CH_3$) was first aroused as a result of its short-lived use as an antipyretic. In demonstrating the remarkable antifebrile effects of this drug, Dreschfeld[5] mentioned that slight toxic effects, consisting of jaundice and hemoglobinemia, were occasionally noticed after repeated doses of from 8 to 12 grains. Although a considerable literature has grown up on the subject, many details of the action of the compound remain obscure.

At the present time it would seem that a compound possessing the properties of phenylhydrazine is indispensable in the treatment of polycythemia, and for this reason precise knowledge of the action of this compound and of its derivatives should be of value. The experiments in the present paper were planned with the object of comparing the effects of phenylhydrazine-hydrochloride and acetylphenylhydrazine when administered in moderate doses over a period of several months. The dosage was such that for considerable intervals the erythrocyte counts were maintained at approximately half the normal values.

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* Hoppe-Seyler conceived the so-called hemochromogen spectrum to be due to a relatively simple substance, $C_{34}O_{35}N_4O_4Fe$. The present view, which is based largely on the work of Anson and Mirsky in Barcroft's laboratory, is that the characteristic spectrum attributed to hemochromogen is given by a conjugated protein consisting of hemoglobin and the base heme, $C_{34}H_{32}N_4O_4Fe$. Synthetic hemochromogens, compounds of heme with a large variety of nitrogenous substances, including hydrazine and phenylhydrazine, give absorption spectra which sufficiently resemble that of the heme-globin compound as to be mistaken for it. For a detailed discussion see ANSON, M. L., AND MIRSKY, A. E.: Physiol. Rev., **10**, 506–546. 1930.

PROTOCOL 1
Rabbit M-1: Phenylhydrazine-hydrochloride

DATE (1931)	ERYTHROCYTES	HEMOGLOBIN	LEUKOCYTES	BASOPHILES	EOSINOPHILES	MYELOCYTES	JUVENILE	"STAB"	SEGMENTED	LYMPHOCYTES	MONOCYTES	DOSE	REMARKS
	millions	grams per 100 cc.	thousands									mgm.	
January 5	4,70	11.36	6,9					2	52	40	6		Weight 3.1 kgm.
8	4,89	11.36	6.1										
12	4,59	11.78	5,5						41	53	6		
13												40	
15	4,21	7.19	10,5		4		1	2	40	49	4		Microcytes, toxic lymphocytes and marked polychromasia
17	3,21	6.53	11,3										
20	2,43	7.45	7,25			1		3	31	57	8		Marked polychromasia, anisocytosis and poikilocytosis
22	3,00	8.34	4,35										
24	3,86	9.62	4,75						42	54	2	20	Weight 3.1 kgm.
29	4,63	10.22	2,75		6				14	76	4		Many toxic lymphocytes
30												50	
February 3	3,05	6.32	12,3	3	1				40	55	1		
10	4,09	9.74	3,7										
11												50	
18	3,98	10.03	3,05	2	4				28	56	10		Toxic lymphocytes, microcytes, Heinz-Ehrlich bodies
19												100	
24	2,43	4.86	19,1	2					36	54	4		Many toxic lymphocytes, Türck cells, Heinz-Ehrlich bodies
March 3	3,70	11.15	5,1	4	2				24	68	2		
5												50	
19													Rabbit delivered litter of six. One died March 31
31	4,00	10.22	7,9										3.2 kgm.
April 4												50	
6												50	
10												50	
14	2,18	5.26	9,3	3		1	1	1	71	22	2		Heinz-Ehrlich bodies
29	4,16	9.02	8,6						44	40	16		
May 5												100	
8	2,27	5.5	11,1						70	30	0		Microcytes, Heinz-Ehrlich bodies
20	5,05	9.77	5,8	2					34	64	0		Autopsy, ether anesthesia

PROTOCOL 2
Rabbit M-2: Acetylphenylhydrazine

Date (1931)	Erythrocytes	Hemoglobin	Leukocytes	Basophiles	Eosinophiles	Myelocytes	Juveniles	"Stab"	Segmented	Lymphocytes	Monocytes	Dose	Remarks
	millions	grams per 100 cc.	thousands									mgm.	
January 5	4,70	11.00	6,9						30	65	5		Weight 2.5 kgm.
8	5,02	11.00	9,1										
12	4,97	11.00	6,75						32	64	4		
13												40	
15	4,38	7.3	9,35						26	71	3		Marked polychromasia, toxic lymphocytes
17	2,78	5.8	11,5										
20	2,53	7.9	7,95	6		2			13	75	4		Polychromasia, toxic lymphocytes, microcytes
22	3,94	8.5	5,6										
24	3,95	9.2	5,15	2					15	80	3	20	
29	4,71	10.22	5,75	4					10	82	4		
30												50	
February 3	3,67	6.9	10,8	3			1	2	21	72	1		Heinz-Ehrlich bodies, microcytes, polychromasia
10	4,37	8.8	6,8										
11												50	
18	3,52	9.7	7,5	2		4			28	56	10		Microcytes, toxic lymphocytes
19												100	
24	2,93	8.0	6,5	2				4	36	54	4		Many toxic lymphocytes, Türek cells Heinz-Ehrlich bodies
March 3	4,46	11.36	3,6	4		2			20	71	3		Weight 2.9 kgm.
5												50	
31	5,60	14.38	6,8										Weight 3.1 kgm.
April 4												50	
6												50	
10												50	
12													Weight 2.7 kgm. Rabbit delivered a litter during the night and devoured it
14	2,84	7.0	9,2	2		2		4	52	38	1		Heinz-Ehrlich bodies
29	6,47	10.4	21,5	4		2		6	9	75	4		
May 5												100	
8	3,33	6.5	25,3	2	1	3		4	36	52	2		
20	4,53	10.1	7,8	1	1			4	34	56	4		Autopsy, ether anesthesia

Twelve rabbits were used in the present series. In Protocols 1 and 2 are summarized the data obtained in two of the experiments, selected for comparison.

Rabbits M-1 and M-2 were under observation for 136 days. One received 0.56 gram of phenylhydrazine hydrochloride, subcutaneously, the other a similar amount of acetylphenylhydrazine. Both were autopsied two weeks after the last dose. Rabbits M-3 and M-4 were under observation for 171 days, during which M-3 received 0.68 gram of acetylphenylhydrazine and M-4 a similar amount of phenylhydrazine hydrochloride. The animals were sacrificed five days after the last dose. Rabbits M-8, M-9, and M-10 were under observations for 277 days. M-8 received a total of 0.5 gram of phenylhydrazine hydrochloride, in small periodic doses, and M-9 and M-10 similar amounts of acetylphenylhydrazine. In each case the last dose was given on the 154th day and autopsies were performed 123 days later.

GENERAL EFFECTS ON THE BLOOD

The destruction of erythrocytes is a prominent feature, but is frequently delayed, except following the administration of very large doses. There is considerable variability in the effect of a given dose of either phenylhydrazine or its acetyl derivative and it is therefore difficult to evaluate their relative actions, but as far as can be judged from the present experiments and earlier observations, the two compounds are approximately equally effective. The increased tolerance to these drugs which various authors have described both in experimental and clinical studies is probably not a property acquired by the organism, but is inherent in the cells. Newly-formed erythrocytes seem to be more resistant to their action than older, more mature corpuscles. If sufficiently long intervals are allowed between injections (four to six weeks), repeated doses seem to have about the same effect.

Of particular interest are the changes in the composition of the erythrocytes. In anemia produced by both phenylhydrazine and acetylphenylhydrazine the volume of the average corpuscle increases, often more than 50 per cent (Bodansky[2]). There is a

definite and disproportionate increase in the water content of the corpuscle (Bodansky and Dressler[3]) and a corresponding fall in its specific gravity (unpublished data), a relative decrease of the hemoglobin (often there is a rise in the absolute amount per cell), and an increase of the non-hemoglobin protein fraction. The average erythrocyte in the experimental anemia produced by these drugs also differs from the normal in containing cholesterol esters and an increased amount of unsaturated fatty acids.

In addition to these changes in chemical composition are the alterations in the physical appearance of the cells. Attention has been called recently by Bratley and associates[4] to the formation of dense, refractile bodies within the cells. These bodies, named after Heinz[11] and Ehrlich[6] have been previously observed, but their significance is not clearly understood. According to Bratley, these "inner bodies" apparently result from a primary condensation of the hemoglobin and the drug, followed by the formation of semifluid bodies which fuse and become semisolid. Heinz-Ehrlich bodies were observed from time to time in all of our experimental animals. Whatever the mechanism for their formation may be, they obviously represent an alteration in the physicochemical properties and distribution of the hemoglobin. The recent observations of Warburg, Kubowitz and Christian[16] bear closely upon this problem. They found that rabbit cells treated with phenylhydrazine and suspended in a suitable, glucose-containing medium, exhibited a ten to twelve-fold increase in respiration and in the capacity to oxidize the glucose. The phenomenon is attributed to the catalytic effect of the hemin which is liberated in the reaction between phenylhydrazine and hemoglobin, the other product of the reaction being globin, as shown by the fact that when the phenylhydrazine-treated cells are hemolyzed, denatured globin separates out. The outward diffusion of the hemin and the retention of the globin probably explains the increase in the non-hemoglobin fraction to which reference has been made. Warburg suggests that the reaction with phenylhydrazine may also affect the young cell at the site of its formation, a point which merits close study. With regard to the increased non-hemoglobin protein content of

the erythrocytes formed during rapid regeneration, it is obviously to be associated with the high proportion of nucleated cells and reticulocytes. The latter may reach very high levels, reticulocyte counts as high as 76 per cent having been encountered in our experiments. That the changes in the constitution of the erythrocytes profoundly alter their physical and chemical properties is to be assumed, but in the further pursuit of the problem, it will be necessary to distinguish the chemical peculiarities of the injured corpuscles from those of the newly-formed and immature erythrocytes.

Long[13] found no relation between leukocytosis and the injection of phenylhydrazine in his experiments with rabbits. On the other hand, Giffin and Allen[1,8] observed a definite, though somewhat irregular tendency toward an increase in the number of leukocytes, and many clinicians have had the same experience in the therapeutic use of phenylhydrazine. In the present series of experiments the majority of rabbits exhibited leukocytosis with considerable regularity. Not infrequently the leukocytosis was followed by a sharp fall to very low levels, at the expense of the granulocytes (for example rabbit M-1, June 29, Feb. 10 and 18). No definite change in the proportion of mononuclears could be determined. The drugs did not produce any very marked shift to the left, as determined by the Schilling count. Myelocytes and even juvenile forms were encountered very infrequently and the stab forms showed only a slight tendency to increase. This observation is of significance, indicating that the appearance of myelocytes and juvenile forms in cases of polycythemia treated with these drugs is probably a manifestation of the disease rather than the effect of the treatment.

ACTION ON THE HEART AND LUNGS

Except for slight acute granular degeneration in rabbits M-3 and M-5, nothing in the microscopic appearance of tissue from the heart could be attributed to the action of the drugs. Rabbits M-1 and M-8 exhibited a small amount of endocardial sclerosis, but were otherwise normal. Rabbit M-10 showed marked fatty invasion of the heart, probably nutritional in origin.

The rabbits which were examined after death by deep ether anesthesia showed edema and marked congestion in the lungs. In the others, the lungs were normal in appearance.

ACTION ON THE KIDNEY

Autopsies were performed on rabbits M-3 (pyrodin) and M-4 (phenylhydrazine-HCl) five days after the final dose. The former showed marked acute degeneration and engorgement; the latter showed extensive degenerative and necrotic changes affecting especially the convoluted tubules. The glomeruli were markedly engorged.

Rabbit M-1 (phenylhydrazine-HCl) and M-2 (pyrodin) were examined at autopsy two weeks after the final injection. Rabbit M-1 showed hydropic changes in the collecting tubules, acute tubular degeneration and extravasation of blood into the glomeruli. Rabbit M-2 showed moderate engorgement and acute degeneration.

Rabbits M-8, 9 and 10 were examined at autopsy 123 days after the drug was discontinued. Rabbit M-8 showed a slight amount of nephrosclerosis; in M-10 there was evidence of a previous degenerative change, and in M-9 nothing unusual was observed.

It appears therefore that both phenylhydrazine and acetylphenylhydrazine are nephrotoxic, the former somewhat more than the latter, and that, if the drugs are discontinued after moderate doses, the damage is largely repaired.

ACTION ON THE LIVER AND SPLEEN

Rabbits M-1 and M-2 showed acute degeneration, of moderate severity, and congestion. M-4 showed marked fatty degeneration and central necrosis; in M-3 the degenerative changes were more limited. The livers of rabbits M-8, M-9 and M-10 were normal and showed no evidence of cirrhosis.

Although in acute intoxications, the spleen may enlarge considerably, in the more chronic conditions, grossly it often appears atrophic. On microscopic examination, the spleens of rabbits M-1, M-2, M-3 and M-4 were found to be congested and to contain abnormal amounts of pigment. Rabbit M-3 showed in

addition a subacute perisplenitis. Rabbit M-8 had a low grade splenitis and in rabbits M-9 and M-10, there was marked fibrosis.

CHANGES IN THE BONE MARROW

The bone marrow of the experimental animals was carefully compared with that of normal animals of about the same age. In M-1, M-2, and M-4 there was evidence of an erythroblastic hyperplasia. In rabbit M-3, the bone marrow was fatty; in M-8, M-9 and M-10 it appeared essentially normal.

ADRENALS AND BRAIN

Bratley and associates have described the occurrence of focal myeloid metaplasia in the adrenal cortex of rabbits poisoned with pyrodin. In our experiments, the adrenals remained normal. This may have been due to the difference in dosage.

Careful examination of the brains of our animals revealed nothing remarkable. In this connection, reference may be made to the interesting observation of Mosse and Rothmann[15] that in dogs made severely anemic with pyrodin, there occurred marked degeneration of the posterior columns of the cord.

SUMMARY AND CONCLUSIONS

1. Acetylphenylhydrazine and phenylhydrazine-hydrochloride produce approximately the same grade of anemia in the rabbit.

2. The apparent tolerance to these drugs which is established is probably not a property acquired by the organism, but is inherent in the corpuscles formed during rapid regeneration. These are more resistant than normal cells probably because of differences in chemical composition.

3. A striking feature of the differential leukocyte count (Schilling) during treatment with these compounds is that there is only a slight tendency toward a shift to the left.

4. Both compounds are toxic, the liver and kidney being especially susceptible to injury. The changes which occur in these organs in chronic intoxications of moderate severity are apparently not permanent, for in animals autopsied several months after discontinuing the drugs, no active lesions were

found, although in two of the animals there was evidence of a previous degenerative change in the kidneys.

5. While a quantitative comparison of the effects of phenyl-hydrazine-hydrochloride and acetylphenylhydrazine in causing tissue damage cannot be made, the impression is nevertheless definite that the latter compound is less toxic than the former.

6. Assuming that a compound of the type of phenylhydrazine is essential in the treatment of polycythemia vera, it would therefore appear that acetylphenylhydrazine is to be preferred to phenylhydrazine-hydrochloride.

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A CHART AND SYSTEM FOR REPORTING AND RECORDING BLOOD EXAMINATIONS

FRED. BOERNER

From the Laboratories of the Graduate Hospital of the University of Pennsylvania; Contributed under the Diagnostic Hospital Endowment

The methods now in common use for reporting and recording blood examinations offer many possibilities of error and misinterpretation which, with our present knowledge of the subject, could be remedied to a remarkable degree. Indeed the more improvement in the method of reporting and recording hematological examinations, the greater will be their value to the clinician with a better and more accurate correlation with other clinical signs.

The commonest causes of error and misinterpretation are well known and the remedy for most of these is simple while other remedies, although simple, appear complicated or radical.

The system for reporting blood examinations discussed in this publication, is simple and yet offers to the clinician a means of interpreting the results with a far greater degree of accuracy and the minimum of mental burden.

In the past too little attention has been given to normal ranges and too much attention to average normals. The average clinician wishes to know when a patient is below or above normal. This can only be answered by a knowledge of the normal range and to him the average normal is of little or no value. He works with high and low normals for blood sugar and has not worried about an exact average normal. The use of the percentage system for reporting results is mathematically unsound and no doubt accounts for many errors in interpretation. For example, a given percentage of hemoglobin can have as high as ten different values when expressed in grams per 100 cc. of blood. The percentages given in all texts for the various types of leukocytes are applicable to normal counts only.

Blood Chart — Graduate Hospital, University of Pennsylvania. Name R T, Room or Ward 217.

Standard Normals and Normal Range*

Examination	Standard Normal (100 per cent)	Normal Range
Hemoglobin (male)............	17.3 grm. per 100 cc. of blood	14-15 grm. (80-105 per cent) per 100 cc. of blood
Hemoglobin (female).........	17.3 grm. per 100 cc. of blood	12-13.5 grm. (70-90 per cent) per 100 cc. of blood
Erythrocytes (male)...........	5 million	4.5 to 6 million
Erythrocytes (female).........	5 million	4 to 5.5 million
Leukocytes	According to age (see below)	4 to 11 thousand
Cell volume (male)............	50 cc. per 100 cc. of blood	40 to 50 cc. per 100 cc. of blood
Cell volume (female)..........	50 cc. per 100 cc. of blood	35 to 45 cc. per 100 cc. of blood
Volume per cent (male).......	Double the cell volume	80 to 100 per cent
Volume per cent (female).....	Double the cell volume	70 to 90 per cent

Normal Range of Leukocytes*

	3 months to 2 years	2 to 5 years	Over 5 years and adults
Neutrophiles	2000-7000	2000-5700	3000-7000
Basophiles	0-50	0-50	0-50
Eosinophiles	15-770	50-770	50-400
Lymphocytes	4000-9700	2500-6700	1700-3000
Monocytes	15-770	25-770	100-500

Normal Index*

15 or over—Normal
10 to 15—Very slight shift to the left
5 to 10—Slight shift to the left
Under 5—Marked shift to the left

*These figures are taken from Approved Laboratory Technic by Kolmer and Boerner, D. Appleton & Co., New York, N. Y., 1931.

REMARKS

11-30-31 No clott retraction
Bleeding time 10 minutes
Coagulation time 8 minutes

12-1-31 Icterus index 6
Van den Bergh direct negative
indirect 0.3

12-9-31 Fragility test 0.44% to 0.30%

For several years I have been devoting attention to this phase of hematology with the result that more acceptable methods have been formulated for reporting leukocyte counts,[1] "shifts to the left"[3] and for calculating indices. These methods have been given several years trial and found very satisfactory and helpful and by their use, the results are much more satisfactory for charting or graphing.

To complete the system a chart* has been drafted which will permit of charting or graphing all the various blood examinations now in common use. The chart was designed with the hope of offering to the clinician a method for charting blood reports which would give some of the advantages that the temperature chart has given. The chart is especially useful in cases of blood dyscrasias and in cases requiring several examinations from time to time. All the blood reports are on one sheet and changes can be noted at a glance. Additional assistance is given by having the normal ranges on the back of each chart together with a space for remarks. The figures given for the ranges are taken from Approved Laboratory Technic by Kolmer and Boerner and which have been discussed in more detail elsewhere.[2]

It is to be clearly understood that the use of the system here described does not in any way improve the accuracy of the various examinations or in any way compensate for the errors in technic or inferior methods used for conducting the tests. However, the system is submitted as a more accurate aid for the detection of changes than the usual methods now in use.

HEMOGLOBIN

The results of the hemoglobin estimations are reported in grams per 100 cc. of blood and indicated on the chart by (x), preferably in red ink. The mark is placed opposite the figure on the scale to which the reading most closely corresponds. If the reading is half way between two of the figures it can be placed on the line dividing them. The two heavy lines represent the low normal ranges, the lower for females and the upper for males. The

* These charts can be purchased from the Keystone Printing Co., 12 S. 10th St., Philadelphia, Pa.

purpose of these lines is to enable the clinician to see at a glance how near or far from normal are the results reported and recorded. It is not expected that these ranges are to be accepted as absolute low normals. They are however, close enough to the usual accepted figures to be of practical value.

The clinician who is still unaccustomed to the gram method and desires to know about what percentage a certain number of grams represents can readily convert the number of grams into percentage by multiplying the number of grams per 100 cc. of blood by 6:

$$6 \times \text{grams per 100 cc.} = \text{per cent of hemoglobin}$$

The exact factor to multiply by to convert grams into percentage so as to correspond to the standard normal is 5.78. The figure given above is close enough however, for practical use and is more convenient.

ERYTHROCYTES

The erythrocyte count is expressed in millions and fractions thereof and is indicated by the mark (o) which is placed opposite the figure on the scale to which it most closely corresponds. The heavy lines represent the low normal ranges, the lower for females and the upper for males (see discussion under hemoglobin).

Various abnormal changes of the erythrocytes commonly reported upon are listed and the degree of change indicated by +1, +2, etc. The reticulated cells are recorded in per cent and the nucleated cells in number appearing in the differential count.

COLOR INDEX

The color index is calculated by multiplying the number of grams of hemoglobin by 3 and dividing by the first two numbers of the erythrocyte count.

$$\frac{3 \times \text{grams of hemoglobin}}{\text{First two numbers of the erythrocyte count}} = \text{Color index}$$

The exact factor to multiply the hemoglobin by is 2.89 to make it correspond to standard normal. However, the figure recommended will give indices very close to those obtained with standard normal and is more practical and convenient.

VOLUME INDEX

The volume index is calculated by dividing the number of packed cells per 100 cc. of blood by first two numbers of the erythrocyte count.

$$\frac{\text{Cubic centimeters of packed cells per 100 cc. of blood}}{\text{First two numbers of the erythrocyte count}} = \text{Volume index}$$

SATURATION INDEX

The saturation index is calculated by multiplying the number of grams of hemoglobin by 3 and dividing by the number of packed cells per 100 cc. of blood.

$$\frac{3 \times \text{grams of hemoglobin}}{\text{Cubic centimeters of packed cells per 100 cc. of blood}} = \text{Saturation index}$$

The exact factor for multiplying the hemoglobin by is 2.89 as stated under color index.

LEUKOCYTES

The total count is not reported or recorded in this system. There does not appear to be any logical reason for including the total count when the number of each type of cell is given and the total leukocyte count so readily computed by adding these together. However, since the use of total counts has become so well established it may be desired by some to have it reported and charted, in which case it can be indicated on the chart by the letter "T." The chart will not show the exact count but will indicate that is between certain figures which is sufficiently accurate for practical clinical purposes.

The number of each type of cell per cubic millimeter is reported and recorded. The various types are indicated by letters, e.g.: "N" for neutrophiles, "L" for lymphocytes, etc. The letter is placed opposite the number on the scale to which the report most closely corresponds, e.g., if the number of neutrophiles is found to be 4,350, the letter N is placed on the line opposite 4-5000. Percentages are omitted entirely as this system of reporting leukocytes is given in all texts, applicable to normal counts only and

therefore difficult to interprete in abnormal counts and is very often misleading.

To obtain the totals for each type separately, it is necessary first to make a total count, then determine the percentage of each type by the differential count. The calculation is made by simply multiplying the number of hundreds in the total count by the percentage of each type separately. The sum total of all types is equal to the total count.

Any report giving the total number of leukocytes and the percentages of the various types can be readily converted into actual numbers of each type by multiplying the number of hundreds in the total count by the percentages.

The normal range for each type, according to age, is given on the back of each chart. The three dark lines on the chart represent the normal ranges for the neutrophiles and lymphocytes. The neutrophiles normally fall between the top and middle lines and the lymphocytes between the middle and bottom lines, so at a glance, any abnormal changes in these cells can be recognized. All of the other types fall below the bottom line according to their range. The basophiles need not be charted or even reported, as the changes in the number of these cells have practically no clinical significance except in myelogenous leukemia.

Abnormal or immature types of leukocytes can be charted by using a suitable symbol.

For reporting and recording "shifts to the left" (Nuclear index) of the neutrophiles, the nuclear index described by the writer elsewhere[2] is advocated. This method is very suitable for determining the degree of shift and simplifies it for the clinician. The index is obtained by dividing the number of mature by the number of immature neutrophiles or metamyelocytes. The normal as well as the significance of changes in the index is given on the back of each chart.

PLATELETS

In cases of purpura the platelets can be charted in the space used for leukocytes, using the leukocyte scale which ranges from more than 200,000 down. The letter P is used for indicating platelets.

TRANSFUSIONS

When a transfusion is given it can be indicated on the chart by x in the space provided and under the proper date. The amount given can be recorded under remarks on the back.

CONCLUSIONS

A system of reporting blood examinations is offered which has the following advantages: (1) A means for reporting and recording hemoglobin in grams with the least inconvenience to the clinician who is only accustomed to percentages. (2) Elimination of average normals and substitution of ranges for interpreting reports. (3) Elimination of the percentage systems for reporting hemoglobin and leukocytes. (4) Elimination of the percentage systems by new and more logical methods for calculating the various indices without disturbing the generally accepted normals. (5) A method for determining "shifts to the left" which is simple yet accurate and very amendable to charting. (6) A chart for recording or graphing the various blood examinations now in common use.

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THE PHOTO-ELECTRIC SCOPOMETER*

WILLIAM G. EXTON

From the Laboratory and Longevity Service of the Prudential Insurance Company of America, Newark, New Jersey

The measurement of transmitted light is a most important and fundamental function of the clinical laboratory because so many determinations depend upon bench manipulations which yield no information at all until the light transmission of the resulting colored solution or turbid suspension has been measured optically. For this purpose a number of different instruments are available which, by one or another modification, make it possible to ascertain the value of an unknown sample by matching its density or brightness with that of a known or standard sample of the same material.

Unfortunately, the instruments which measure color best do not measure masses in suspension so efficiently, and vice versa. It is therefore necessary for laboratories to keep on hand nephelometers and turbidimeters as well as colorimeters.

Furthermore, many biological materials such as hemoglobin, protein, indican, et cetera are either so troublesome to isolate, so scarce, or keep so poorly that their routine preparation as standards for use in colorimeters and nephelometers is hopelessly impracticable. To meet these deficiencies it is necessary to resort to a multiplicity of cruder devices which feature more or less approximate substitute standards for special determinations. Notwithstanding their lack of precision, laboratories must also have some of these on hand. Thus the cost of acquisition and upkeep as well as the necessary training for operating the variety of instruments now in use are disadvantages which laboratory directors are incessantly hoping and trying to overcome.

* Read before the Tenth Annual Convention of the American Society of Clinical Pathologists, Philadelphia, Pennsylvania, June 7–9, 1931.

Light transmission measurements are still made visually, although it is well understood that all visual measurements suffer from ineradicable aberrations and limitations which are inherent in optical physiology. As an instance, the very limited range over which Bier's law holds is not always appreciated. It is also well known that some colors are more difficult to match than others; in fact, many people never succeed in matching certain colors with confidence. It is common experience that lower and higher concentrations are measurable only in a haphazard approximate way, if indeed at all, because of too much glare in the lower and too little light in the higher densities.

Furthermore, there are not only individual differences in vision but the same person cannot always count on consistent vision, because even slight or temporary changes in mental or physical condition may affect it. In short, the human eye is not only defective but the brain back of it is also subject to physical and psychical variations. Thus, even under ideal conditions with perfect instruments and the utmost skill, visual measurements are subject to uncontrollable errors which arise from a number of different causes.

Since the discovery of conditions which translate light proportionally into electricity, many efforts have been made to eliminate the errors inseparable from visual measurements by substituting for human eyes the photo-electric effect or electric eye, as it is so often called. The theory is so perfect that it is not at all surprising to find the literature rich in descriptions of photo-electric measuring devices. On the contrary, it is rather more surprising to find that photo-electric instruments for clinical laboratory work are not yet available. The failure is, of course, attributable to practical difficulties resulting from the inconstancy of light sources, the limitations of photo-electric tubes, especially fatigability and low energy output and, in my opinion, also to the photo-electric practise of measuring light in terms of electric units.

In a previous paper I tried to show how efforts which have hitherto been made to overcome these basic difficulties ended in complicating matters with amplifying tubes and circuits, current control devices, potentiometers and other meters, and batteries

with other accessories needing temporary as well as permanent connections. As such arrangements cannot be molded into finished, self-contained instruments, they are necessarily awkward and even when operated with the utmost skill and vigilance subject to sudden deviations from standardization without warning. They are, therefore, unsuitable for the conditions which obtain in most clinical laboratories where accuracy and consistency depend so much upon the simplicity of operation and stability of measuring instruments.

In the same paper I also described experiments intended to adapt the photo-electric effect to scopometry[1,2,3,4] by trying to avoid rather than complicate the basic practical difficulties. Thus the inconstancy of light sources was balanced out by using the simplest possible classical Wheatstone bridge circuit which has nothing in it to adjust. In conjunction with this, another experiment designed to measure light transmission photo-electrically in terms of light rather than in the terms registered by electric meters, which have hitherto been used for photo-electric measurements, was also described. It was found that a combination of these principles greatly reduced the limitations of photo-electric tubes by permitting the employment, as a means of measurement, of the differential between two similar tubes. In this way amplifying troubles were found avoidable and the double advantages of a null method (electrically and optically) and maximum photo-electric tube performance gained.

The success of these experiments led me to construct two instruments which have been in continuous operation four to six hours every working day over periods of three and two years, respectively, by twenty or more individuals engaged in doing the routine and research work of the Prudential Laboratory. These instruments have proven so stable, reliable and efficient that I have ventured to entrust the Bausch & Lomb Optical Company of Rochester, N. Y. with making the Photo-Electric Scopometer available to others.

DESCRIPTION

Figure 1 shows the general appearance of the instrument, which is a self-contained unit without batteries or accessories ready for operation when con-

nected with the usual house current. It will be seen that two symmetrical optical paths run from opposite sides of the lamp house to two similar photo-electric tubes. In the left path there is a recess for the specimen cell, and in the right there are interposed two adjustable diaphragms: an iris or setting, and a rectangular or measuring diaphragm with attached speedometer scale. The apertures of both diaphragms are controlled by fine adjustments similar to those on microscopes. Directly in front of the observer and clearly visible at the same time are the galvanometer dial with its zero point slit and the scale from
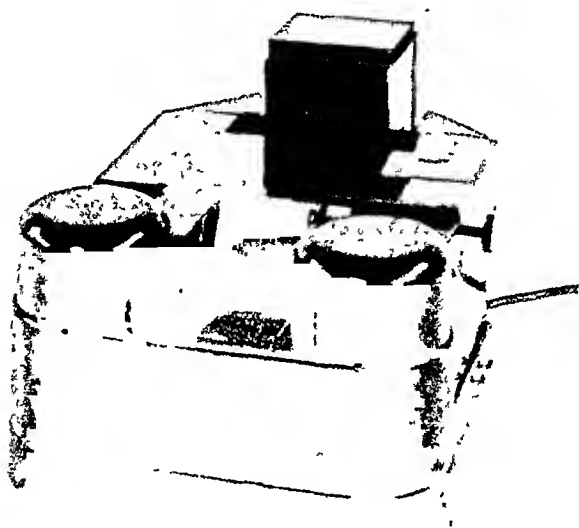
FIG. 1. ELECTRO SCOPOMETER

The optical paths lead from the lamp house to the photo cells in the front of the instrument. Note the recess on the left for the specimen; on the right the button for turning the measuring diaphragm with the setting iris-diaphragm behind it, and the switches for bridge and lamp conveniently placed together in the base.

which measurements are read. The optical system in its dust and smoke-proof housing is designed to distribute parallel light equally to both photo-electric tubes.

The electric system (fig. 2) comprises a lamp and a bridge circuit with independent switches placed conveniently together on the right side of the instrument. The lamp circuit is the usual type and the bridge circuit is the simplest conceivable, consisting of only wire and three fixed resistances. The circuits are wired hard and fast as in a radio and there is nothing in them to adjust or get

out of order. The other electric parts are the photo-electric tubes, the galvanometer and the lamp.

The photo-electric tube is, of course, the heart of any photo-electric measuring device. There are many different kinds on the market, but I have experienced the greatest difficulty in securing tubes which I considered efficient for photometry. In fact, I feel greatly obligated to Dr. H. C. Rentschler, Research Director of the Westinghouse Company, for his interest and skill in providing special tubes for the scopometer which answer every clinical pathological necessity and which he is able to reproduce so very closely. The color sensitivity of Dr. Rentschler's tubes leans a little more to the blue end of the spectrum than does the human eye, but they are more than red sensitive enough to measure efficiently
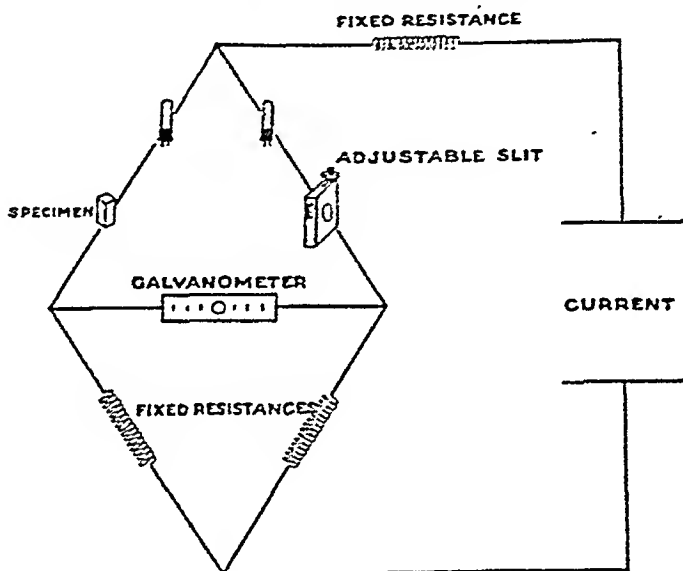
FIG. 2. PHOTO-ELECTRIC SCOPOMETER CIRCUIT

Note that all electrical connections are permanent and there are no electrical adjustments of any kind.

hemoglobin and the other yellow, red and brown compounds of clinical pathological interest, without filters of any kind. They are free from fatigue and dark current effects and, above all, they are so sensitive to turbidities that they measure the slightest by transmitted light better than do nephelometers or tyndallmeters by reflected light. The photo-tubes are housed in light, dust and smokeproof compartments, and when firmly in place need no care at all. In fact, they should not be handled, because moisture from the hands may cause surface leaks (which, however, can be cured by wiping and drying the surface with alcohol). Three of the four photo-tubes in our original instruments are still in operation, one pair for more than three years, and up to now no deterioration or other perceptible change is apparent. Our experience, therefore, supports the theo-

retical assumption that well-made photo-electric tubes are permanent in life and function.

The suspension and magnet of a stock galvanometer, which is truly described as being simple and sturdy, are integral parts of the instrument, and I am grateful to the Leeds and Northrup Company of Philadelphia for providing a special suspension which is abundantly sensitive for the photo-tubes that are the standard equipment of the scopometer. Like the tubes, the galvanometer needs no attention and should not be handled. It does not require leveling, and notwithstanding the wear and tear of three years' continual use and the abuse of repeated transportations in all kinds of vehicles and cutting in and out of many experimental setups, the original galvanometers still function perfectly without ever having needed any kind of attention.

A stock 250 watt projection lamp is the standard light source of the instrument, and when free from defects, these lamps can be changed at will without affecting calibration.

The standard specimen cell has plano-parallel sides 6 millimeters apart and an extended outside edge for convenience in handling and protection against finger smears. Three cubic centimeters suffice to fill it.

OPERATION

There are two preliminary steps to operating the Photo-Electric Scopometer, i.e., (1) checking galvanometer and (2) setting instrument for the desired determination. (1) To check the galvanometer: switch on the lamp (bridge circuit off) and turn the suspension head of the galvanometer (under the sliding cover in front of the dial) or tilt the instrument to one or another side until the galvanometer rests at zero. (2) To set the instrument for a particular technic: Place the specimen cell holding clear water or any predetermined standard in its recess and set the measuring scale on zero (by turning the button projecting from the right side of the instrument). Then turn on the bridge current and bring the galvanometer back to zero by changing the aperture of the iris or setting diaphragm which is controlled by the fine adjustment in back of the measuring scale.

Both checking and setting are delicate but extremely simple manipulations which need only be done when starting work or changing from one technic to another. However, they take only a moment, and it is well to be sure that the galvanometer registers zero when inactive.

Measurement of unknown samples is exceedingly speedy and convenient. With a specimen in place all one has to do is to turn the button which changes the aperture of the measuring diaphragm until the galvanometer again rests at zero and refer the indicated scale reading to a reference graph or table showing the equivalents of concentrations and scale readings.

The scopometers employ predetermined calibrations in place of the usual standard samples and calculations required in pho-

tometry. This presents no difficulties but rather offers a number of practical advantages such as freedom from troubles and inaccuracies incident to preparation and control of comparison standards, a very considerable increase in speed and convenience of operation, and the ability to measure materials which are too
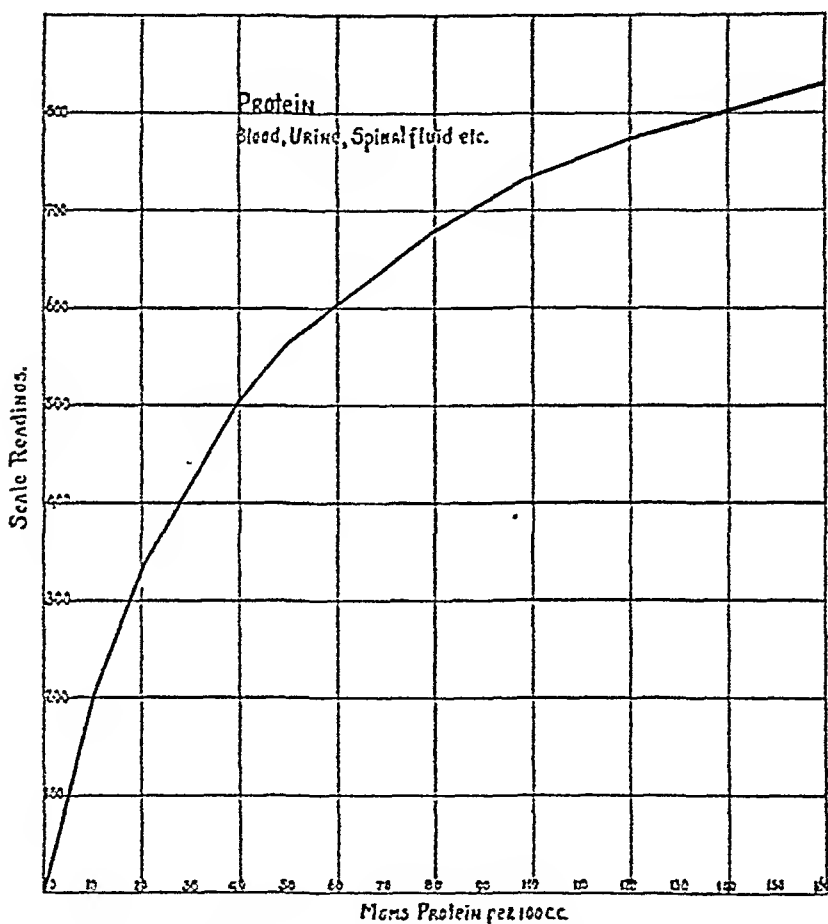

FIG. 3. PROTEIN CALIBRATION OF PHOTO-ELECTRIC SCOPOMETER II

The protein concentrations are plotted against Photo-Electric Scopometer scale readings (Jour. Am. Med. Assn., 80: 529).

scarce or otherwise impracticable for the preparation of comparison standards.

Illustrative calibrations in the form of graphs are shown in figure 3 for protein in blood, urine and spinal fluid, et cetera, in figure 4 for blood sugar by several different methods, and in

figure 5 for hemoglobin by iron and acid hematin methods. A calibration is made by applying any method to a series of known concentrations covering the desired range and plotting the
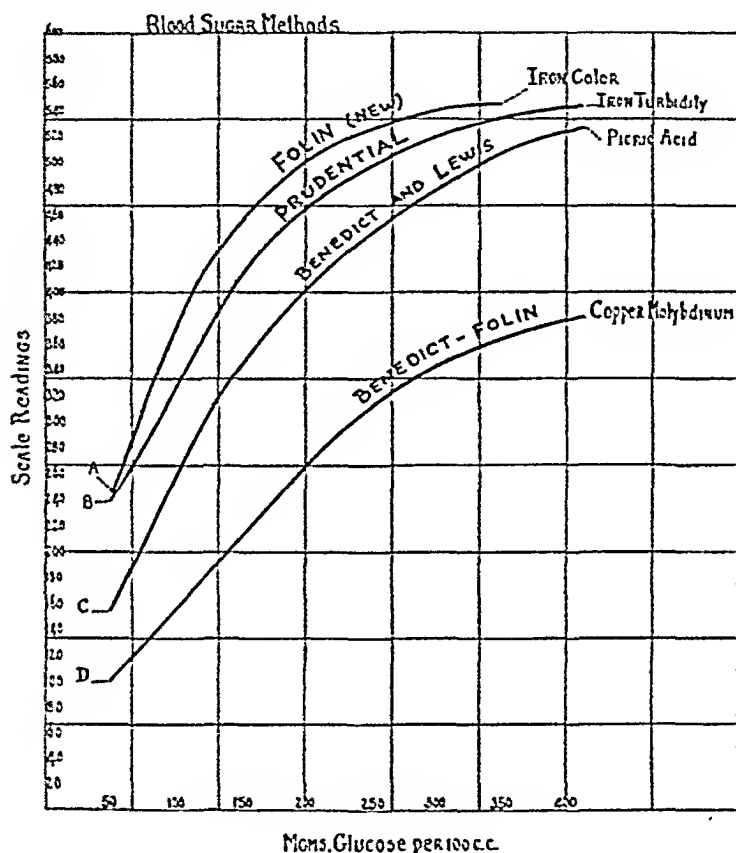
FIG. 4. BLOOD SUGAR CALIBRATIONS OF PHOTO-ELECTRIC SCOPOMETER II BY FIVE DIFFERENT METHODS

Sugar Methods. (A) Blue colloidal suspension (Jour. Biol. Chem., 77: 421). (B) Light yellow turbidity (Trans. Med. Dir. Assn., 1929). (C) Red solutions (Jour. Biol. Chem., 20: 61). (D) Blue solutions (Jour. Biol. Chem., 70: 405; 76: 457).

scopometer scale readings against the concentrations. Thereafter the calibration need never be made again because the scale readings indicated by subsequent similarly treated samples are always referable to the original calibration. Specimens may also

be measured without a pre-determined calibration by adjusting a known standard sample to give the same scopometer reading as the unknown sample. Very critical titrations may similarly be made.

It is, of course, important to measure tests at the right time, which is generally stipulated in the descriptions of methods, but if there is any doubt about the time a reaction takes to reach the
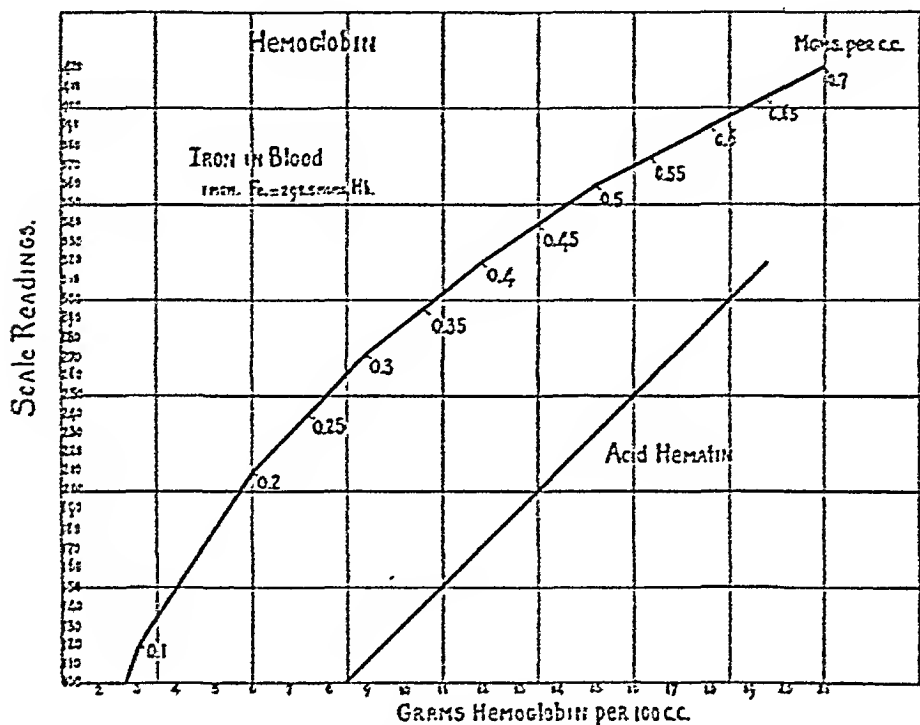
FIG. 5. HEMOGLOBIN CALIBRATIONS OF PHOTO-ELECTRIC SCOPOMETER II

Calibration for the pink and red-brown colors obtained with two different hemoglobin methods.

period of its greatest intensity and stability, it is a simple matter to complete a test on an intermediate concentration and measure it at successive time intervals. In this way crystal and bacterial growth, enzyme action and phenomena like precipitation, agglutination, and lysis may also be measured.

Experiments in measuring the same materials with seven different pairs of similar photo-electric tubes indicate that when the best attainable accuracy is not essential, calibrations will

hold within 5 per cent with different instruments. In the course of an early experiment one of the original tubes was accidentally damaged and another tube substituted for it without apparent effect on calibrations. In another experiment, three novices and an experienced observer measured with the same instrument a series of nine serum protein precipitates running from 10 to 80 mgm. per 100 cc., checking within a quarter of one per cent. Thus, by totally eliminating personal and subjective sources of error the instrument enables the least competent technician to measure as accurately as the most skillful worker.

Chemists and technicians who have used the instrument for experimental and routine work agree that they can measure with it more accurately than they can do the bench work prescribed for determinations. They also emphasize that those who have not had an opportunity to work with the Photo-Electric Scopometer cannot appreciate its ease and speed. Other advantages include the use of micro-samples, extraordinary range and great sensitivity, especially in the lower ranges where sensitivity is most useful.

SUMMARY

A universal self-contained fool-proof instrument which measures the light transmission of macro- and micro-samples photo-electrically in terms of light has been described and the null principles which underlie its remarkably easy and precise operation discussed. A three-year test has proved the sturdiness and reliability of the Photo-Electric Scopometer and demonstrated its superiority over visual methods in range, sensitivity and accuracy.

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(3) Exton, Wm. G.: The Junior Scopometer. Jour. Am. Med. Assn., 92: 708-713. 1929.
(4) Exton, Wm. G.: The present status of clinical laboratory measurements with note on photo-electric effect. Am. Jour. Clin. Path., 1: 237-250. 1931.

EDITORIAL

On the Control of Cancer

'Midst all that is talked, written and done about the control of cancer, there is surprisingly little mentioned of what might be called the ideal of a cure.

Every sympathy is extended to those who must treat the many patients with the disease and appreciation is expressed of the emotions of the patients and their relatives and friends who grasp at every straw in the desperate situation of hopeless cancer. But there must nevertheless be something toward which practising physicians, patients, relatives and in fact every one should look as an ideal sometime to be attained.

When surgery and radiation no longer offer any hope of cure or amelioration and recourse is had to narcotics alone as an easy means of bridging an interval, it might be just as well to again emphasize ideals which should already have been discussed when surgery and radiation were contemplated at the first consultation.

Not that it is not said that "some day, someone will find something;" and even more definitely, "some day someone will find out how to immunize people against it;" or "some kind of chemotherapy will conquer it" and so on.

But what have we on which to base a rational program, on which to plan an ideal?

Some things are known well enough.

(1) All living multicellular organisms increase the number of their cells by a process called cell division.

(2) As the cells increase in number, they are differentiated and organized into the morphology and function characteristic of the species and the parts.

(3) The number, differentiation and organization of cells produced in the majority of species is limited by the organism itself, not only in the total primary cells, but also in those produced

secondarily for repair, regeneration, et cetera. An equilibrium is reached.

(4) In tumors of whatever kind, the number of cells is increased. The equilibrium is disturbed in degrees from slight to complete. Differentiation and organization are diminished in degrees from slight to complete.

(5) There are authentic cases on record in which even malignant tumors have been held in check or have even disappeared through agencies within the organism itself. Many more cases are known, in fact are encountered every day, in which recurrence of tumors has been checked from within for many years after removals which have failed to remove all of the cells.

From these few facts alone, rational plans for future control are obvious. There seems no need to state that when control of inanimate nature is accomplished, it is usually by way of physics and chemistry.

STANLEY P. REIMANN.

NEWS AND NOTICES

ELEVENTH ANNUAL CONVENTION OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

May 6 to 9, 1932, New Orleans, Louisiana

The Eleventh annual meeting of the American Society of Clinical Pathologists for the presentation of scientific papers was held in the Jung Hotel, on May 6-7-8-9, 1932. The meeting was attended by fifty members and twenty-seven visitors. The program proved to be of unusual interest to all those present. This program was made notable by an excellent group of papers dealing on the general subject of Hematology and Serology.

The address of the retiring President, Dr. H. J. Corper, was read at the annual banquet and this was followed by an excellent paper on "The Pathology of Amebiasis in Carriers" by Col. Chas. Craig. Greetings from the American College of Surgeons was extended by Dr. M. T. MacEachern and greetings from the American Medical Association were extended by Dr. William D. Cutter.

A feature of the meeting was the dedication of the Department of Pathology, of the Louisiana State Medical School.

The business meeting was called to order at 9:40 A.M., May 9th, by the President, Dr. H. J. Corper.

In addition to the items previously reported in the JOURNAL, the following reports were read and approved. In some instances the report is abridged.

REPORT OF THE SECRETARY-TREASURER

Despite the depression our income from dues this year exceeded the previous year by $320.00. Due to rigid economy in all expenses the income exceeded the total expenses by $114.00, in spite of the fact that the journal costs in excess of one-half of the membership dues. It is obvious that with such a small net income the society must consider carefully any new activity which would call for funds from our treasury. Since the society is now successfully engaged in a

major activity (the publishing of its official journal which has gained for it worthy scientific recognition), it would seem desirable that a certain portion of our cash balance be set aside as a permanent endowment fund for the increase of the scope of the journal. I would suggest that the society at this time set aside $2000.00 from its present assets for this purpose.

The membership is to be congratulated on the fact that approximately 50 per cent of the total dues collected were paid within a month after the bills were sent out. It is only by prompt attention to such seemingly small details that much expense can be saved in handling the affairs of the society.

Your Secretary again wishes to bring to your attention that no member will receive the journal until their dues are paid. As soon as the payment is made, $5.00 of it is sent to the Publisher of the journal. Thus, when dues are allowed to lapse the name is removed from the mailing list of the journal. The reinstatement on the mailing list usually requires sometime and occasionally errors occur as evidenced during this year. Therefore, if you wish to receive your journal without interruption, please pay your dues on receipt of the first statement. If the journal is not received promptly after paying dues, please notify the Secretary.

To date the active membership of the society is 356. Three hundred and six of these have paid the current year dues. Thirty-eight owe for one year and thirteen owe dues for two years. During the past year six members resigned their membership in the society. One of these members resigned for financial reasons, but hopes to be reinstated as soon as his financial condition warrants it. Three members abandoned the field of pathology to engage in the field of internal medicine. Thirteen members were dropped for non-payment of dues and no replies to inquiries could be obtained from them even after the usual registered letter. Eighteen members who owed two years of past dues have now fully paid and are awaiting reinstatement by the Board of Censors. Two members died.

BALANCE SHEET—APRIL 20, 1932 AND STATEMENT OF INCOME & EXPENSE FOR
PERIOD FROM MAY 19, 1931 TO APRIL 20, 1932

ASSETS

Balance in bank (Willet, Fink & Wharton Report)............................		$3,257.17
Investments:		
Commonwealth Edison Company First Mortgage 4 per cent Gold Bond— Par Value $1000.00—Cost........		945.00
March 1, 1932 Interest Coupon Attached.......................		20.00
Furniture and fixtures....................	$601.75	
Less-Reserve for Depreciation.........	460.86	140.89
Total assets.....................		$4,363.06

NET WORTH

Balance—*May 19, 1931*...............		$4,248.82
Income:		
Initiation Fees................	$230.10	
Membership Dues..............	3,710.00	
Interest on Investments........	25.33	
Miscellaneous.................	1.00	
Total....................	$3,966.43	
Expense:		
Salary.....................	$525.00	
Office Expense................	27.70	
Printing and Multigraphing......	123.95	
Technicians' Registry..........	422.00	
Postage.....................	115.00	
Cartage.....................	6.00	
Convention Expense...........	316.36	
Williams & Wilkins.............	2,127.91	
Audit......................	42.50	
Bond.......................	12.50	
Taxes (Colorado).............	12.77	
Depreciation on Fixtures........	120.35	
Exchange...................	.35	3,852.19
Income in excess of expense...		114.24
Net worth April 20, 1932.....		$4,363.06

As you will note the society is in excellent financial conditions, having a cash balance of $3,257.17 and a $1000.00 Commonwealth Edison Bond.

<div align="right">A. S. GIORDANO, <i>Secretary-treasurer.</i></div>

REPORT OF EXECUTIVE COMMITTEE

On recommendation of the Editor of the American Journal of Clinical Pathology, the committee urges that the members give their support, by personal or institutional subscription, to Biological Abstracts.

Recommend that the Society authorize the Secretary-Treasurer to set aside the sum of $2000.00 as a special JOURNAL fund.

Recommend that the Society express its appreciation of the excellent business and editorial management of the JOURNAL by the Editor, Dr. T. B. Magath.

Recommend that an appropriation of $500.00 be made for the expenses of the editor's office.

Recommend that the Society extend to the Publication Committee and to Dr. Kolmer and his co-editors their thanks and appreciation for the excellent character of the book on "Approved Methods of Laboratory Technique."

The committee examined the report and accounts of the Secretary-Treasurer

and found the financial conditions of the society in a highly satisfactory condition, as will be determined in his reports to the Society, and commends the secretary for the excellent and economical administration of this important office.

K. M. LYNCH, *Chairman.*

BOARD OF REGISTRY

We now have a total of 1055 names on our Registry of which 1009 are laboratory technicians and forty-six medical technologists. Two hundred and seventy-one registrants (two hundred and fifty-three laboratory technicians and eighteen medical technologists) were added during the fiscal year and eight names were stricken off the roster.

The growth of the Registry has been quite constant. Hospitals are now demanding our certificates from applicants for positions.

There has been marked improvement in the quality of technicians applying for registration. This has been partly effected by raising the standards of admission in the training schools approved by our Registry which now require a year of college work in chemistry and biology prior to entrance and a minimum period of instruction of twelve months. The members of the Board are making plans for a more rigorous selection of registrants by instituting examinations, oral and written, theoretical and practical.

It is interesting to note that through circularizing our registrants one hundred and nineteen copies of Drs. Kolmer and Boerner's "Approved Laboratory Technic" have been purchased through our registrar.

In a number of the larger cities technicians are beginning to band themselves into local societies for the advancement of their interests. Their aims are chiefly intellectual. At their meetings they have invited clinical pathologists to address them on topics relating to clinical pathology. The registry has encouraged such organizations as they tend to elevate the scientific status of their memberships. Possibly in the not distant future and through their own efforts a national organization of Laboratory Technicians may be effected similar to the one in the nursing profession which will still further enhance the prestige of a calling so essential to the clinical pathologists and so necessary in the human health program.

The Board of Registry has received during the past year a total of $3,877.29 and has expended $2,249.00 leaving a balance on hand of $1,628.29.

PHILIP HILLKOWITZ, *Chairman.*

Report on training schools for technicians

Numerous inquiries have been received in reference to the training of technicians. We have furnished from time to time, a list of the names of the schools for laboratory technicians, approved by the Board, through registration.

The applications of three hospital laboratories were approved, two others and one from a university were provisionally accepted and one rejected during the present session.

Nearly forty schools are now on the list of approved schools, through registration, including several university and college courses in medical technology and the courses offered in hospital laboratories.

We wish to encourage the members of this body who conduct a training course for technicians in their hospital laboratories to have it registered with our Board by meeting the minimum requirements as outlined in our previous report.

Evils of the commercial schools are increasingly too evident to be ignored by the clinical pathologist. It is his duty to counteract these evils by insisting on the minimum standard requirements for the school for technicians and to lend active support to every endeavor to elevate the qualifications of laboratory technicians and the standards of the training schools, through registration with this Board.

KANO IKEDA, *For the Committee.*

REPORT OF THE PLACEMENT BUREAU

The committee wishes to report that during the past year there was received three applications for change of positions and one of these was filled through the help of the bureau.

The committee urges every member that desires a change of location to place his name on file with the committee so that as soon as openings occur the members can immediately be notified.

A. S. GIORDANO, *Chairman.*

REPORT OF THE EDITORIAL COMMITTEE

The American Journal of Clinical Pathology began its second year under very auspicious circumstances. The enclosed letter from the publishers clearly indicates the condition and shows that there is no indebtedness resulting from the first year's activities. This is an unusual showing for it means that we have been able to absorb the initial expenses that ordinarily would take three years to write off. By careful planning, it is obvious that during this year we shall be able to have a more liberal policy concerning the acceptance of illustrations for articles and it may be that the JOURNAL will show a profit.

During the first year the Executive Committee voted to pay for an excess of fifty-two pages but the income from the JOURNAL met this expense and hence it will not have to be borne by the Society. A large number of manuscripts has been submitted to the JOURNAL and these together with other matters had necessitated a considerable amount of correspondence on the part of the editor, resulting in more than one thousand letters being written to transact the business of the JOURNAL.

The JOURNAL is now being abstracted in all of the principle abstracting journals in this country and abroad. It has gained considerable recognition and there is no reason to doubt that the scope of the JOURNAL can be materially increased in the future.

Owing to the good showing during the first year, the publishers have voluntarily agreed to increase the size of the JOURNAL by fifty pages for this year. The number of subscriptions has reached nearly one thousand and when this happens another one hundred pages will be added to the JOURNAL.

Not a small amount of credit for the success of the JOURNAL is due to manufactures advertising in the JOURNAL, and I again urge members of the Society to express their appreciation in whatever way they see fit to our advertisers who in reality have contributed a goodly sum of money to the JOURNAL.

T. B. MAGATH, *Chairman.*

REPORT OF PUBLICATION COMMITTEE

The book on Approved Laboratory Technic has been apparently received in the United States and abroad in a very highly satisfactory manner. A large number of reviews have been published and all have been satisfactory and highly commendatory so that the authors are highly gratified with its reception and particularly since it reflects to the credit of the Society.

The authors also wish to take this opportunity of asking the members of the Society to bring errors to their notice so that these may be corrected in future editions. A reprint of the book appeared early in April and a number of errors were detected in time for correction.

The Committee also wishes to refer with great satisfaction to the success of the American Journal of Clinical Pathology which is largely due to an editor of exceptional ability and a splendid coöperation of the publishers, Williams & Wilkins Company of Baltimore. Indeed we feel that the editorial policy of this JOURNAL has placed it on par with the finest being published and the Society can take great pride in this publication.

JOHN A. KOLMER, *Chairman.*

REPORT OF RESEARCH COMMITTEE

Your committee wishes to report some progress during the year, but regrets that in the assembling of the hematologic registry there has been considerable delay on the part of some of our members in sending in their case reports. About fifteen cases with slides were sent in during the year, most of which were received within the last two weeks. Over an equal number which are of little or no use to the Society were reported without accompanying slides.

Only two men reported their experience with the Friedman test for pregnancy, but as was brought out at the meeting it is hoped that all the members will send in returns on a questionnaire to be sent out soon in order to assemble the data to be tabulated as a commentary to the papers delivered on the subject by Dr. Sondern and Dr. Reinhart.

No data were received in regard to hemoglobin determination according to Osgood and Haskins technique, although fifty-nine men obtained standard

solutions from Dr. Osgood. We trust that we may be able to hear from these men in the near future. We are pleased to report that the Ward Burdick medal was awarded to Dr. Benjamin Kline.

We believe that with participation on the part of our members to a greater degree than in the past, we may be able to assemble information and material available to all members of the Society, which can never be obtained in any other way, and plea for better coöperation with future committees.

A. G. FOORD, *Chairman*.

REPORT OF COMMITTEE ON MEDICAL AND HOSPITAL FUNCTIONS

It was moved and seconded that in view of the difficulties in financing exhibits at medical and hospital associations that this committee be abolished.

C. H. MANLOVE, *Chairman*.

PUBLIC RELATION COMMITTEE

The first meeting of this Society was held with the avowed purpose as stated by the temporary chairman, Dr. Hillkovitz, "to take council how best to strengthen the status of the Clinical Pathologist from both a scientific and an economic standpoint—to analyze our weakness and to institute proper measures for insuring the stability of our specialty, a member of which is unfortunately looked upon as a technician rather than a consultant." With the help of the A. M. A. and other organizations our standing as specialists is now well established. It remains for the individual to advance his field of usefulness and appreciation by physicians, by the brand of work he puts out and by his activities in local and state societies and in hospital staff meetings. Unfortunately however, the same economic problems exist practically unchanged as they did in 1922, namely:

(1) The state laboratory.
(2) Competition of lay hospital laboratories with private laboratories.
(3) Status of approved private clinical laboratories of approved hospital laboratories and approved clinical pathologists.
(4) Competition by technicians.
(5) Relation of the pathologist to the hospital.

THE STATE LABORATORY

There is today unquestionably a drift toward state or socialistic medicine fostered by lay individuals, industrial organizations, insurance companies, and the lay press which seems destined to grow, due in part to the lethargy of the medical profession. The policies of many state board of health laboratories in extending their activities foster and encourage this tendency. Clinical pathologists, who are most seriously affected, because they are, so to speak, in the

front line trenches, should, if they want to continue their existence accept this vanguard position, and individually and collectively resist to the full extent of their ability, this invasion, which will ultimately include the whole medical domain. When the public finds out it can have its laboratory work done at state expense, why should it not look to the same source for its calomel and quinine and its surgery.

One of our members from one state submits evidence in the form of a full page advertisement, of their State Board of Health, in their State Journal which "offers to every Physician Free Laboratory Service of all kinds, offering also free biologicals," and says this pernicious procedure if persisted in will unquestionably stifle all private laboratories in that state.

That the situation is not hopeless can best be answered by citing the results of our endeavors in Indiana, where conditions have been particularly bad. Your chairman in 1928 wrote and had introduced before the House of Delegates a resolution (copy attached to this report), asking the state society to go on record as condemning the socialistic activities of the state board of health, in the following points:

(1) Entering into unfair competition with licensed physicians, making clinical pathology a specialty.

(2) Forcing the expense of private laboratory work on the tax-payers under the guise of Public Health.

(3) That their work should be confined to epidemiology, to indigent patients, and to state institutions. This resolution was referred to the committee on Public Policy and legislation who the following year reported at length (copy of report attached), giving their impressions of the situation. They recommended that the House of Delegates ask the laboratory men of the state to appoint a committee to meet with the state board of health, discuss their problems and report the results back to the House of Delegates. This was adopted and accordingly on August 6, 1930, a committee consisting of Drs. Giordano, Rhamy, Lyons, Langdon, and Forry met with the state board of health. With the backing then, of the state medical society and of the state journal, we succeeded in having them adopt the following proposal:

"That there be printed upon the cards enclosed in Wassermann containers sent out by the state laboratory and requiring the doctor's signature the following statement:

"'This patient is financially unable to pay for this laboratory service and I am making no charge for said service,' also that there be printed upon these cards in bold face type, and requiring the signature of the patient: 'I am financially unable to pay for this laboratory test and I know the state laboratory charges no fee for said laboratory test.' Also upon these cards shall be printed unless the signature of both the physician and the patient appear in proper place, 'this test will not be made by the state laboratory.'"

Supplementary to this understanding an opinion had been obtained from the

Attorney General to the effect that "under the law it was not mandatory that the state laboratory make examination for those who were able to pay, except, and only in case of epidemics, and to preserve community Health."

A report of this meeting and its results was made to the House of Delegates who then officially approved the proposed method. Information cards as agreed upon are now in use in Indiana, as approved by the Indiana State Medical Society (see Indiana State Med. Jour., Oct. 1930). The responsibility of keeping faith now lies squarely on the shoulders of the Secretary of the State Board of Health.

Such a program in every state would go far towards protecting the rights of the Clinical Pathologist. We recommend that the A. S. C. P. officially approve this step on the part of the Indiana State Medical Society and the State Board of Health and by some means urge its acceptance in principle and practice by all state boards.

Therefore the following resolutions are recommended for your approval:

(1) Resolved: That this society express its approval of the action of the Indiana State Medical Association and the State Board of Health of Indiana in requiring that the examinations of their public health laboratories be limited to public institutions and to indigent patients, who must state in writing as well as their attending physician, that they are unable to pay for such laboratory examination.

(2) That a copy of this resolution be sent to the American Medical Association, all state associations and state boards of health, calling attention to these facts and of our approval of the same.

(3) That a copy be sent to all councillors and that they be urged to bring this matter before their state associations and endeavor to have this plan adopted in their respective states.

(4) That full details of this plan be printed in our own Journal for reference.

The arguments used in accomplishing this result in Indiana are as follows:

(1) Extension of the activities of the State Board of Health to all branches of medicine is the road to state medicine.

(2) Encourages unscrupulous physicians to have laboratory work done at state expense, charging the patient and pocketing the fee.

(3) Encourages pauperism on the part of the laymen.

(4) Curtailment of their activities would reduce taxes. Tax-payers should be impressed with the fact that thousands of dollars of state funds are annually diverted for the benefit of private individuals fully able to pay their way.

(5) The value of the community of a fully equipped laboratory in competent hands.

HOSPITAL LABORATORIES

Improvement of laboratory service in hospitals should be encouraged. In many places clinical pathologists have connection with more than one hospital

and this method for the smaller hospitals should be strongly urged by the A. M. A. council.

The present situation as regards standardization of hospitals needs stimulation for betterment. The tendency of lay hospitals to commercialize their laboratory by entering into competition with private laboratories for outside work is wrong in principle.

Although it is realized that in communities where private laboratories are not available, the hospital laboratory must be utilized, yet it should be a cardinal principle that hospital laboratories should not enter into such competition with private laboratories, for by so doing they are invading the domain of medical practice. The clinical pathologist in charge of a hospital laboratory is usually on salary and does not share in fees thus collected, and this amounts to commercializing him at the expense of his fellow pathologists. This competition is most unfair to both the hospital pathologist and the private laboratory, especially when the outside work is done at special hospital prices. This is a growing evil and some remedy should be found. As an instance, one physician in the same office building as your chairman stated he would rather have his work done close at hand if the hospital laboratory prices would be met. Too often the hospital, instead of being a place where physicians take patients for better care and treatment under his order, is in active competition with one or more branches of medicine, and dictate to the physician instead of being subservient to him. It would be a good thing if all laboratories whether private, institutional or public health should be classified and rated. We note that the council of the A. M. A. has "shifted the emphasis in listing from approved laboratories to a more inclusive list of Physicians Specializing in Pathology and allied subjects, whether they are in teaching, hospital, research, governmental or commercial laboratories." Just what the effect of this change will be remains to be seen, but on the surface it seems like a withdrawal of their support of the clinical laboratory. A much more beneficial plan would be to make separate classifications of approved private clinical laboratories, approved hospital laboratories, laboratories for research, et cetera.

TECHNICIANS

Efforts should be continued to have all technicians registered and classified according to their qualifications and to curb their employment in situations not under proper supervision.

B. W. RHAMY, *Chairman.*

REPORT OF NECROPSY COMMITTEE

It is the understanding of this committee that it owes its existence to the unquestionable expression of opinion during the past two years that tissue pathology should receive more prominent consideration in this society in the

future; and it is the belief of this committee that this sentiment is quite universal in the society because it is apparent that the majority of the members, regardless of their hobbies and special interests, are fundamentally pathologists whose daily work includes autopsies and the examination of surgical material.

The one constructive and tangible thing which this committee has to offer is a set of rules which have been formulated dealing with the proper preparation and care of the body after death and during and following autopsy. These rules are designed to eliminate some of the objectionable procedures which have been openly condemned by this society and to institute procedures and precautions which will gain better repute for autopsy work with the laity, the profession in general and the undertakers. These rules, which are at present not type-written, will be submitted to the officers and, it is hoped, the publication committee will see fit to have them published in the official JOURNAL. It is not to be understood that by publishing such rules this committee is attempting to dictate autopsy procedure to pathologists, but they are intended to be used for guidance by professional and lay assistants in the less technical details of routine autopsy work.

(1) This committee believes that any educational campaign or propaganda directed toward the laity had best be handled by the national necropsy committee.

(2) That the chairman of the necropsy committee of this society should automatically be this society's representative on the national necropsy committee.

(3) This committee believes that autopsy demonstration by nationally prominent pathologists should occupy a permanent place on the program of the annual meeting of this society. Such demonstrations could either be scheduled on Sunday or at the time of the meeting of the Executive Committee when the vast majority of the members are at leisure.

O. A. BRINES, *Chairman.*

REPORT OF NECROLOGY COMMITTEE

Whereas we have lost by death two of our members, Dr. W. F. Thompson of Beaumont, Texas and Dr. George Henry Fox of Binghamton, New York

Be it resolved that we express our sincere sympathy to the families of these two departed colleagues.

Obituaries

George Henry Fox was born at Painted Post, New York, in 1882, and received his premedical education at Detroit University and Amherst College. He was a graduate of the University of Michigan Medical School in 1909. He was a past president of the Broome County, New York Medical Society. He died of cerebral hemorrhage on December 26, 1931.

William F. Thompson was a charter member of the American Society of Clinical Pathologists. He was a graduate of Tulane University. For many years he was secretary-treasurer of the State Pathologic Society of Texas.

HARRIET J. LAWRENCE, *Chairman*.

AMENDMENTS TO THE BY-LAWS

The following changes were approved:

Article III, SECTION 1.

Shall be changed to read as follows: All Fellows and Associate Members shall subscribe to this Constitution at the time of their election to membership and shall pay an initiation fee of Ten ($10.00) Dollars, payable with the application for membership.

Article IV, SECTION 6.

Add the following: Whose duty shall be to foster research by Fellows of the Society. They may suggest problems for investigation, foster group investigations, and collect and maintain catalogues of data and materials.

Article V, SECTION 1. At each annual session the Research Committee may designate a Fellow of the Society to receive the Ward Burdick Award. This award shall be in the form of a gold medal which shall be presented to that Fellow who, in the opinion of the Research Committee, has presented the most meritorius contributions to the science of clinical pathology. Rules governing the award shall be made by the Research Committee, approved by the Executive Committee and published for the information of Fellows of the Society. If, in the opinion of the Research Committee, at any annual session no contribution is judged of sufficient merit to receive the award, no award shall be made at that session.

The insertion of the above article as number V automatically changes the number of each subsequent article number.

The incoming President, Dr. Walter M. Simpson moved that the President be given the privilege of appointing the following committees for the coming year: Public Relations Committee, Committee on Local Arrangements, Necrology Committee, Committee on Necropsies.

The motion was voted upon and passed.

The chair of the President was formally turned over to Dr. Walter M. Simpson by Dr. H. J. Corper and being no further business the meeting adjourned at 12:45 P.M.

RESOLUTION PASSED BY THE COUNCIL OF THE MINNESOTA STATE MEDICAL ASSOCIATION, JUNE, 1932

WHEREAS, Both the Radiological Society of North America and the American Society of Clinical Pathologists, through their

respective subsidiary agencies, namely, the American Registry of Radiological Technicians and the Board of Registry of Technicians, are endeavoring to raise and maintain the minimum standards of educational and technical qualifications of technicians in their respective fields, through certification and registration, and

WHEREAS, The American Medical Association, the American College of Surgeons, the American Hospital Association and other responsible national medical organizations have given their unqualified support to this program of certification and registration by the aforementioned Societies, therefore,

Resolved, That the Minnesota State Medical Association, through its Committee on Schools for Laboratory Technicians, extends its hearty coöperation to these Societies in their efforts to elevate the standards of qualifications of medical technicians by recommending to its constituent members and County and District Societies that they urge the clinical laboratory and X-ray technicians under their influence, to identify themselves with the national registry through registration.

Resolved, That the Committee on Schools for Laboratory Technicians of the Minnesota State Medical Society proposes, for the present, at least, to recognize only those institutions which enjoy the approval of the American Society of Clinical Pathologists through registration or recognition and withhold its recognition from all others, pending such registration and approval.

SOME ESSENTIALS FOR SATISFACTORY WORK IN ALLERGY*

J. H. BLACK

Dallas, Texas

Within recent years it has been shown conclusively that (1) allergic conditions are quite frequent and are found in a considerable part of the population, and (2) that many individuals may be relieved by proper diagnosis and treatment. This has led to a marked revival of interest in these conditions and many men have already entered or are contemplating entrance into this type of work. This, I believe, justifies setting forth a few well-defined essentials which should be understood by those undertaking the care of such persons. There are, of course, a vast number of technical details and bits of information that one acquires in this work which serve to increase the skill and knowledge of the worker. Many of these may be learned by perusal of the literature. I wish to call to your attention in this discussion a few necessities which must be acquired before satisfactory work may be done.

First essential: Ability to differentiate between allergic and non-allergic conditions.

It should be self-evident that treatment on an allergic hypothesis of a non-allergic person is useless. But it should be pointed out that differentiation is not always easy. Patients not infrequently present themselves with a diagnosis of bronchial asthma whose dyspnea is due to cardiac weakness. It is not always easy to determine whether a patient is suffering from a hay fever or an acute coryza. A child was sent to me with a diagnosis of an allergic nasal condition which proved to be a leutic periostitis of the nasal bones. One must know as much as possible of general

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medicine in order to avoid mistakes of this kind. A good practice, I think, is to consider every patient non-allergic until he is proved to be otherwise. This may cause the loss of an occasional patient but it will prevent some unhappy moments.

Second essential: Some knowledge of the mechanism underlying allergic manifestations.

Even a casual survey of the literature will convince one that a vast amount of knowledge is lacking but there are certain facts and theories which form a basis for this study and ignorance of these leaves one carrying on his work empirically and with no rational basis on which to build. Every one engaged in this work should be able to contribute something to its growth by his own observations. It cannot be done by one who has acquired only the technical skill for the necessary procedures.

Third essential: A working knowledge of the various substances which may act as allergens.

It certainly is important to know the botany of the locality in which one works. One must know what grasses, weeds and trees are found, their relative abundance, the time of pollination and their botanical relationships. The five postulates of Thommen should be kept in mind: (1) the pollen must contain an allergen, (2) the pollen must be wind-borne, (3) the pollen must be produced in sufficient quantity, (4) the pollen must be sufficiently buoyant to be carried a long distance, (5) the plant producing the pollen must be widely and abundantly distributed. Lack of knowledge regarding these factors makes for failure in treatment.

Environmental substances, some of which are occupational dusts, must be known. Animal emanations and dusts of various kinds occur under many and diverse circumstances and the more information one has regarding these substances the better the results of treatment.

Even a knowledge of foods and their preparation is a prime requisite. A patient may be so sensitive to egg white as to react to the small amount found in most baking powders. If one does not know that baking powders commonly contain egg white, failure with this type of patient may result. The removal of a given substance from the dietary is not complete until all foods

containing even a small amount of the substance are removed. Unless one knows something of the composition and preparation of foods, good results will be infrequent.

Fourth essential: Willingness to devote time to a painstaking history and examination.

In no other field of medicine is this more important. Allergy is not a special isolated phenomenon which can be cared for without regard for the rest of the patient. Allergic conditions are definitely affected by the psychic state, endocrine disturbances and autonomic nervous system reactions. A careful investigation into all the possible interrelationships may be necessary and nothing is of more value than a detailed history. In many instances the allergen may be discovered only by good "detective work" and infinite patience is a prerequisite.

Fifth essential: Ability to interpret skin reactions.

There has been the impression, fostered by some commercial institutions, that the diagnosis of the etiologic factor in allergy may be made easily and certainly by skin tests. This, unfortunately, is frequently untrue. Skin reactions are a valuable aid in diagnosis but they are subject to error and slavish dependance upon them will often lead one astray. Reaction to a given substance may occur in the absence of clinical sensitiveness to that substance or be absent in spite of definite clinical sensitiveness. Skin reactions should be correlated with the patient's history and knowledge of his environmental and dietary relationships. A young physician who had examined a patient with hay fever asked me to supply him with two pollens for treatment. One of them was a pollen which is believed never to cause hay fever, the other was one found in the air at a season entirely different from that in which the patient had his symptoms. Both of these pollens may have reacted but some other pollen caused the hay fever of the patient.

There are advocates of both scratch and intradermal testing. Because of the danger of severe reactions it is my custom to use intradermal tests only after scratch tests have been made and found negative. A positive reaction to scratch testing does not need corroboration by intradermal technic. A negative reaction

by scratch test may be checked by intradermal injection with safety. This method causes some duplication of effort but it is both efficient and safe. Scratch tests are frequently not effective and intradermal tests, without preliminary scratch tests, may be unsafe. The character and amount of reaction may differ considerably with the scratch and intradermal technic. Considerable experience with both methods and careful observation are necessary to evaluate reactions.

Sixth essential: Recognition of multiple sensitization as the rule rather than the exception.

In the past many of us have been guilty of examining patients sufficiently to find one substance reacting which might be correlated with the history and stopping all diagnostic effort at that point. The knowledge has been forced on us that allergic individuals are usually sensitive to more than one thing and they have the tendency to become sensitive to other things which may become of clinical importance if the exposure of the patient to the allergen is sufficient. The examination should be inclusive enough to cover all possible factors relating to the condition which the patient presents.

Seventh essential: Potent extracts which deteriorate slowly must be used, some definite standard of measurement must be adopted and, in treatment, the dosage must be individualized.

There are various methods of preparation of extracts. Of these, various modifications of a glycerin saline solvent are most generally used. The preparation of extracts is quite simple and satisfactory solutions may be purchased or made.

There is no certainty at present as to the chemical nature of the allergenic substance so there is no entirely satisfactory standardization. The three methods in use are based on the nitrogen content of the extract, the amount of allergen in the solution expressed in units, and the amount of allergen in the solution expressed in terms of percentage of solute to solvent. Any one of these methods may be used if the user is acquainted with it and recognizes its limitations.

It should be perfectly evident that treatment must be individualized and routine dosage cannot be followed. Some patients

require much smaller increments in dosage than others and some require that treatment be carried to a much higher level to obtain protection than do others. Unthinking adherence to a printed schedule of dosage may lead to failure or over treatment.

Eighth essential: Knowlecge of action and dosage of various medicinal agents.

In addition to specific treatment of allergy the management of patients in acute attacks and the use of non-specific agents in the control of the allergic state are important.

It is important to know that epinephrin may be used in large doses and that morphine may be very dangerous, that children frequently require epinephrin in doses as large as those given to adults and that some drugs which may be effective in relieving attacks may be contraindicated because of a hypersensitiveness to the drug. Many details, which may not need consideration in other conditions, do demand attention and the ability to use properly the various medicinal agents is essential to the comfort and even the safety of the patient.

Ninth essential: It is important to know how to advise patients so they may secure good results and to know what may be promised in the way of cure.

While the results of accurate diagnosis and careful treatment have improved year by year, it is evident that the intelligent, continued coöperation of the patient is necessary and this is best secured by a frank, full discussion of treatment and what may be expected from it. It is further evident that whether the patient may be promised prompt and complete relief, delayed but sure cure, or only a partial amelioration of symptoms depends upon several factors which must be known if a correct prognosis is to be made. Many patients are disappointed because of over-enthusiasm and lack of knowledge which cause the physician to promise more than he can do.

Tenth essential: Great patience on the part of both physician and patient.

Treatment of many allergic individuals is not a matter of days but of months and even years. Even after the patient has been told of the long, continued treatment required, a certain number

become discouraged and need support. A physician who wants to see results at once and who cannot possess his soul in patience had better shun work in this field. A friend once said to me that no one would do this type of work but an old maid. Whether the terminology is correct or not the idea of meticulous attention to details and continued effort along the same line is correct.

These ten essentials by no means exhaust the list of requirements for satisfactory work in allergy. There are multitudinous details which go to make up the art and science of care of allergic patients. But the points discussed are necessary if one is to undertake this work, in a competent manner. Satisfactory work in allergy does not require any unusual order of intelligence but it does demand an enthusiasm and willingness to work which will carry one over a good many disappointing days which are sure to appear.

MYELOID IMMATURITY IN PERNICIOUS ANEMIA[*]

FRANK J. HECK

Division of Medicine, The Mayo Clinic, Rochester, Minnesota

In the morphologic study of pernicious anemia the macrocytosis of the erythrocytes plays such a striking part that relatively little attention is paid the leukocytes, other than to note leukopenia or relative lymphocytosis. Next in significance to the macrocytosis is the presence of hypersegmented polymorphonuclear neutrophils, or neutrophils showing pathologic hyperpolymorphism. Besides these changes from the normal, there may occur, particularly in the severer degrees of anemia, immaturity in cells of the myeloid line back to the stem cell or myeloblast. Although the number of immature cells is seldom great enough to throw doubt on the diagnosis, occasionally a case will be seen in which the immaturity is so marked that, at least temporarily, a diagnosis of myelogenous leukemia is suggested. The observation of such a case resulted in this study of the incidence of myeloid immaturity in a series of sixty-five cases of pernicious anemia.

The literature contains a number of reports on this point, but in many of them the data are incomplete and determinations of hemoglobin or erythrocyte or leukocyte counts are not given. Ziegler[10] reported a case in which there was 4 per cent neutrophilic myelocytes, which increased to 14 per cent following infusion of salt. Naegeli[6] stated: "I find myelocytes in the majority of cases ($\frac{1}{2}$ to about $1\frac{1}{2}$ per cent). Their occurrence is a capricious one. According to former theories, they are signs of severe marrow affection and signify in no way the beginning of a myeloid hyperactivity." In the severer degrees of anemia, Zadek[9] found myelocytes present more frequently than absent, and in his study of 200 cases he was able to demonstrate as high as 6 per cent

myelocytes without necessarily having an increase in the percentage of metamyelocytes above that of the myelocytes. Eight of twenty-two cases studied by Hittmair[4] showed myelocytes; in five of the eight there were promyelocytes and in four, myeloblasts. Schauman and Saltzman[5] found normal myeloblasts in 50 per cent of their cases but only at the height of the disease, and they also mentioned the transitory nature of these observations. Anderson[1] stated that Downey found myeloblasts in a case he reported. Neuburger,[7] Jedlička and Beránek,[5] and Held[3] among others reported on the immaturity. Arneth,[2] however, vigorously denied their presence and stated that in his cases even at the height of the disease and just before death, myelocytes could not be demonstrated.*

Zadek rightly emphasized that in considering the morphologic picture of pernicious anemia at any particular time, one must take into consideration whether the patient is in beginning relapse, at the low point of any particular relapse, or in beginning remission, and must consider the rapidity of remission, since all these affect in varying degree, the blood picture from the standpoint of leukocytes. This also holds true in the study of the erythrocytes, for in the very early stages of pernicious anemia or in the late stages with the disease in an aplastic phase, macrocytosis may be absent.

The material for this study was selected at random by the laboratory technician, without regard for the differential count, but a larger number of cases among the more anemic group were examined. The differential count was made on 500 cells, this number being arbitrarily chosen. With a few exceptions I made all determinations. The table shows that it is likely in the ordinary differential count of 200 cells, immaturity might easily have been missed in some cases. By the same token it would be fair to assume that some of the cases without immaturity would have shown it if 1,000 or 2,000 cells had been counted. The terminology used is that of Pappenheim, so that myelocytes and

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* N. Rosenthal has called my attention to a case reported by Brill (Tr. Assn. Am. Phys., 30: 547–561. 1915) in which, following aplenectomy for pernicious anemia, leukocytosis of 73,000 developed with as high as 11.9 per cent neutrophilic myelocytes and 8.5 per cent myeloblasts.

promyelocytes correspond to the mature and immature myelo-cyte of other authors, whereas leukoblast and myeloblast are both included under the heading of stem cell or myeloblast by the same authors.

For purposes of classification cases have not been considered as showing immaturity unless myelocytes were seen. On this basis twenty-five of the sixty-five cases (including one case reported in greater detail later) fall into this group; in nineteen of these leukoblasts or myeloblasts were demonstrated; in five pro-myelocytes, and in one case only, myelocytes. In some instances

TABLE 1

DIFFERENTIAL BLOOD COUNT IN A CASE OF PERNICIOUS ANEMIA

	1-6-30	1-14-30	1-15-30	1-16-30	1-17-30	1-18-30	1-20-30	1-21-30
Hemoglobin*	22	28		30		40		40
Erythrocytes†	1.05	1.39		1.64		1.85		2.07
Leukocytes	3,100	22,600	11,500	9,200	6,200	5,000	8,800	7,100
Neutrophiles	75.8	37 2	47.8	64.8	66.0	66.0	77.2	82.4
Lymphocytes	20.8	9.0	9.0	6.4	10.6	14.8	16.2	10.4
Monocytes	1.8	5.2	11.0	9.2	12.6	7.4	2.8	4.4
Eosinophiles	1.2	5.2	6.8	4.8	5.4	7.4	2.6	2.8
Basophiles		0.4		0 2		1.2	0.2	
Metamyelocytes		14.2	8.2	8.4	1.6	0.6		
Myelocytes		11.6	10.0	2.8	2.0	2.0	0.8	
Promyelocytes	0.2	13.0	4.6	3.4	1.8	0.6	0.2	
Leukoblasts	0.2	3.6	2.0					
Myeloblasts		0.6	0.6					
Reticulocytes	0.1	14.2		19.4	21.4	10.6	9.3	11.2
Volume index	0 99	1.31		1.33				
Blood urea‡	72§	38		30	28	32	24	

* In per cent. † In millions. ‡ Mgm. per 100 cc. § 1-9-30.

only one or two of the more immature forms could be found, and in others they were present in relatively generous numbers. Leukoblasts or stem cells were not found in any case with an ery-throcyte count higher than 2,030,000, but in fifteen cases in which erythrocyte counts were lower than this, immaturity could not be found. The point emphasized by both Neuburger and Zadek that metamyelocytes may not be found in as large numbers as myelocytes or promyelocytes is supported by my observations. Four cases have been studied at intervals of one to four days to

determine how rapidly the immature cells disappear from the peripheral circulation when the patient is placed on treatment of one form or another. In two cases in which intravenous injection of liver extract was employed all immaturity had disappeared by the nineteenth day. When oral treatment was used the immaturity disappeared within approximately the same time (the twentieth day). After the institution of treatment there appears to be a temporary increase in the amount of immaturity, but this rapidly disappears with clinical improvement.

Because of the unusually marked reaction in one case, a table of details has been arranged (table 1).

REPORT OF CASE

A man, aged fifty-four years, came to The Mayo Clinic January 3, 1930 complaining of weakness and loss of sensation in his feet. In February, 1929, he had consulted his home physician because of swelling of feet and hands associated with numbness and tingling, intermittent diarrhea and sore tongue. His case had been diagnosed as pernicious anemia and liver extract and whole liver had been prescribed. Improvement followed, and during the summer of 1929 the erythrocyte count was almost normal. Failure to carry out the diet conscientiously resulted in relapse of symptoms.

The skin was pale and lemon yellow. The tongue was smooth and atrophic. The spleen was definitely palpable. There was slight edema of the hands and feet. The blood pressure in millimeters of mercury was 158 systolic and 106 diastolic; the pulse rate was 82 beats each minute and the temperature 97°F. The patient was sent to the hospital and January 4, 1930, the concentration of hemoglobin was 20 per cent, erythrocytes numbered 1,010,000, and leukocytes 1,800 in each cubic millimeter of blood. Free hydrochloric acid could not be demonstrated in the gastric contents at the end of one and a fourth hours. Urinalysis was negative. Roentgenograms of the thorax January 4 and 9 gave negative results. Fluoroscopic examination of the stomach was negative. January 7 he was placed on a diet containing ½ pound of raw swine stomach each day. During his stay in the hospital his temperature varied from normal to 100.6°F. and January 8 he was delirious most of the day. The concentration of urea at this time was 64 mgm. in each 100 cc. of blood. Five hundred cubic centimeters of 10 per cent glucose in 1 per cent saline solution was given intravenously and this was followed January 9, 1930, by a transfusion of 500 cc. of whole citrated blood which caused a chill and rise in axillary temperature to 102°F. On this date the concentration of urea rose to 72 mgm. in each 100 cc. of blood. The urine contained pus graded 2, and 60 cells to the field. This was the only time during the patient's stay in the hospital that the urine was found to be abnormal. January 8 the treatment had been changed to Lilly's liver extract 343, four vials being given on that day; this was increased to eight

vials on subsequent days. A rapid drop in the retention of urea occurred, so that by January 13 there was only 32 mgm. of urea in each 100 cc. of blood. After this date the concentration of urea on one occasion reached 38 mgm., but at the time of the patient's dismissal January 29 it was 24 mgm. in each 100 cc. of blood.

An infectious process could not be found to account for the slight fever, and after January 16 the temperature never rose above 99°F. The thorax, abdomen and upper part of the respiratory system did not afford positive data, and at the height of the leukocytosis, January 14, a roentgenogram of the thorax showed it to be normal. From January 10 on there was rapid improvement in symptoms and January 20 the liver extract was reduced to six vials a day.

The first blood smear was examined January 6. Morphologically there was no macrocytosis, although the cells were well filled with hemoglobin. This is in agreement with a volume index of 0.99 on that day. The neutrophils, however, showed increased segmentation. There was little evidence of regeneration in the erythrocyte line and five counts of reticulated erythrocytes from January 4 to 10 showed a maximal of 0.1 per cent of these cells. The next smear examined was taken January 14, at the height of the leukocytosis. By this time there was marked macrocytosis with many polychromatophilic cells. The numerous immature cells gave the appearance of chronic myelogenous leukemia, although the macrocytosis was so marked that there seemed to be little doubt about the diagnosis. The increased segmentation in the neutrophils persisted during this time. January 13 and 16, volume index determinations were 1.31 and 1.33, respectively, so that this conformed to the change in appearance of erythrocytes. The rapid return to normal differential count corresponded to the clinical improvement.

Eight days elapsed between examination of the first two smears, and it is possible that the maximal reaction may have been missed. Since on January 11 the leukocytes numbered only 2,500 it is unlikely that there was ever much more marked reaction than on January 14. What part the retention of urea in the blood played in bringing about this reaction, if any, is difficult to determine.

Zadek reported that in a rapidly occurring spontaneous remission in pernicious anemia he had observed a leukocyte count of 20,000 with an increase in immaturity at that time. Remissions in pernicious anemia induced by modern methods of treatment are probably essentially not different from those occurring spontaneously when considered from a morphologic standpoint except for the more rapid recovery and return to a much higher hemoglobin and erythrocyte level than usually occurs spontaneously.

In the morphologic differential diagnosis an occasional case of early acute or chronic leukemia may be seen in which there is mild hyperchromatic macrocytosis, and in which the amount of

immaturity has not yet reached a degree sufficient to make a positive diagnosis. If the patient is suffering from pernicious anemia, adequate treatment will soon cause disappearance of the immaturity instead of the increase which would occur in the usual course of acute or chronic leukemia.

SUMMARY

In nineteen of sixty-five cases of pernicious anemia, leukoblasts or stem cells could be demonstrated in a differential count of 500 cells; five cases showed promyelocytes and in one case only myelocytes were found.

As a rule immaturity occurs primarily in cases in which erythrocytes are below, 2,000,000, although in fifteen cases in which they were below this point immaturity was not found.

After the institution of modern treatment there may be an increase in the amount of immaturity within a short time. This is occasionally quite marked.

In the four cases observed for a considerable period, the immaturity had disappeared by the twentieth day.

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THE HEMATOPOIETIC SYSTEM AND INFECTION*

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Every pathological process, to be understood, must be considered from its physiological source. In discussing leukemias or any of the so-called blood diseases we must consider the normal or physiological process with which the blood cells and the hematopoetic system are concerned.

All blood cells are derived from a stem cell belonging to the reticulo-endothelial system which is found all over the body, particularly in the bone marrow, liver, spleen and lymph nodes. The erythrocytes and granulocytes (leukocytes) are formed in the bone marrow, the agranulocyte (lymphocyte) is formed in the lymph system, and still a third white cell which is neither granular nor agranular, called the monocyte, has its origin in the reticulum cells of many organs. (See fig. 1.)

These cells are constantly being produced by their respective formative tissues, function in their individual capacities, and are then destroyed. There is therefore a constant and perpetual production and destruction of blood cells occurring in the body as a physiological process. While some of these cells are probably formed in the reticulo-endothelial system of the spleen, it is this organ together with the lungs which is particularly concerned with destruction of the aged cells. It is quite conceivable that these two processes, the manufacture and destruction of leukocytes, are so related as to balance each other and maintain an equilibrium which is compatible with health. If there is a body demand, such as occurs in infection, for an increased number of leukocytes the production process is stimulated and the formative organs expel a greater number of such cells into the peripheral

circulation as evidenced by an increased leukocyte count. After the demand is met the destructive process is stimulated, the leukocytes in excess are destroyed and the normal equilibrium between these two processes is again established as evidenced by reduction of leukocytes to within normal limits. Similarly some such mechanism must be operative in the maintenance of a normal number of erythrocytes in the peripheral circulation.

Normally the leukocytes are subject to only slight and temporary variation in number such as may be caused by muscular exertion or digestion. With the introduction however of any foreign substance into the body, as illustrated by infection, the forces representing the body defense mechanism are immediately stimulated. Foremost among these forces is the reticulo-endothelial system which supplies the body demand by an increased supply of leukocytes or other cells needed to combat the interference with the normal cell balance. The blood changes in pathological conditions may, on this basis, be due to the same processes of production and destruction as occur in normal metabolism but in an exaggerated form. That is, an increased leukocyte count in disease may be explained as resulting from an increased cell formation accompanied by a proportional decreased cell destruction.

The pathological condition underlying any of the so-called blood diseases is found in the blood forming elements and not in the blood; we must then consider aplastic anemia and agranulocytosis as diseases of the bone marrow, Hodgkin's disease as a disease of the lymphatic system and the leukemias as diseases of the general hematopoetic system. It is universally accepted that aplastic anemia and agranulocytosis are almost always complicated by severe gangrenous inflammations located on some mucous surface. These two conditions are in some way related and differ pathologically only in the extent of involvement found in the formative tissues. In aplastic anemia the entire hematopoetic system of the bone marrow is involved and the granulocytes, erythrocytes and blood platelets are greatly reduced in number. In agranulocytosis only the granulocytes are greatly reduced or absent while the erythrocyte count is normal or just
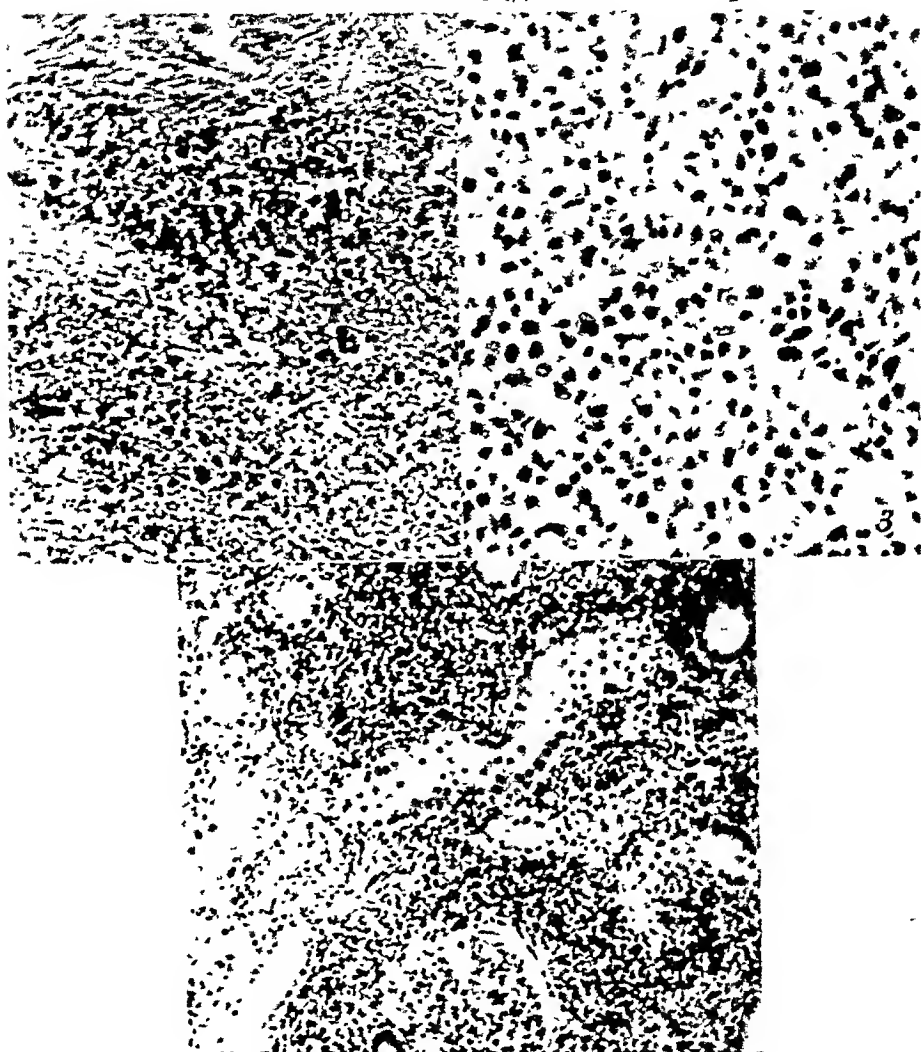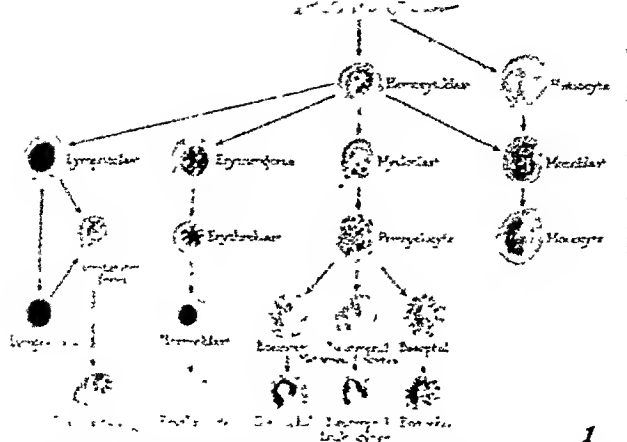
FIG. 1. NORMAL CELL DERIVATION
After R. H. Jaffé

FIG. 2. VARIED CELLS OF HODGKIN'S DISEASE

FIG. 3. MYELOGENOUS LEUKEMIA OF SPLEEN
(Various forms of granulopoesis)

FIG. 4. LYMPHATIC LEUKEMIA OF KIDNEY
Infiltrations pushing aside the tubules

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below normal. In both conditions, however, gangrenous inflammations are encountered as complications. It may be assumed that some abnormal body constituent accounts for the lack of proper activation of the hematopoetic system when the body demand is increased as, for instance, in infection.

Similarly we must concede that hemorrhagic tendencies are indeed characteristic of both inflammation and some of the diseases involving the hematopoetic system. Symptomatic purpura hemorrhagica is secondary to many septic processes and is almost always found in certain types of endocarditis. Coke[2] definitely supported this theory of sepsis in purpura and Lovett[6] isolated *Pseudomonas aeruginosa* (B. pyocyaneus) from throat lesions with which he reduced the granulocyte count in rabbits.

Hodgkin's disease, variously classed between pseudo-leukemia and lymphosarcoma, presents a histological picture which varies from hyperplasia in the early stage to fibrosis in the late stage. As the disease progresses, however, a rather characteristic picture is seen: marked hyperplasia of lymphoid cells, numerous plasma cells, endothelial cells, eosinophiles and many very large multinuclear giant-cells. These cells, while not in formation of the usual inflammatory process, seem to favor infection rather than new growth as their basic origin (Fig. 2). It has long been recognized that a close relationship exists between tuberculosis and Hodgkin's disease and that a similar relationship exists between the leukemias and infection is quite plausible.

Because of the trinity idea of the three types of leukocytes, leukemias are accordingly classified into the three corresponding types: (1) myelogenous leukemia or myelosis with existing pathology in the bone marrow, (2) lymphatic leukemia or lymphadenosis with existing pathology in the lymph system, and (3) monocyte leukemia or reticulosis with existing pathology in the general reticulo-endothelial system.

In the clinical interpretation of the leukemias, there is limited evidence of any definite etiological factors. The fact that many cases of leukemia seem to begin with the sudden opening of some focus of infection favors the infectious theory of etiology. Ellerman[3] demonstrated quite clearly the infectious nature of leu-

kemia of the fowl and maintained that the causative organism is a filtrable virus. While these findings are not yet applicable to man there is considerable evidence that the leukemias are infectious in origin. Ewing[4] stated that he had seen leukemic processes develop from pneumonia and acute tonsillitis. Jaffé[5] was quite firmly convinced that leukemias are infectious in origin and cited cases of acute leukemias, especially in the young, following, the opening of a chronic focus of infection as for example by extracting an abscessed tooth. He further reported a case of a peculiar form of septicemia in which there was an active proliferation of the reticulo-endothelial cells and suggested the term spetic reticulosis.

It is rather interesting also to note that as ordinary infections produce an increase in the number of leukocytes in the peripheral circulation, meta-myelocytes and the younger forms of mature leukocytes are increased in number. The more severe the infection the younger are the mature leukocytes and in very severe infectious processes even immature cells may be seen in the peripheral circulation. It is on this basis that the various indices, such as have been described by Arneth[1] and Schilling are established. In the leukemic pictures also these younger mature forms are greatly increased in number even though the true immature cells predominate.

In the pathological interpretation of the leukemias as demonstrated by the microscopical examination of the hematopoetic tissue there seems to be no difference between acute and chronic forms and no essential difference between leukemia and aleukemic leukemia. In all leukemic cases whether chronic, acute or aleukemic the essential findings are marked proliferation of immature cells in either the bone marrow or the lymphatic system, and formation of immature hematopoetic tissue in the liver and spleen. An explanation of the aleukemia, offered by Szilard,[7] is that these immature cells are very fragile and break down as soon as they enter the peripheral circulation.

In a section of the spleen in myelogenous leukemia, for instance, great accumulations of granulocytes are seen (fig. 3). These cells are in various forms of immaturity (myeloblasts

to mature myelocytes) and indicate the various forms of granulo-poesis. Many of these cells are large with large irregular nuclei and present a picture that may resemble that of malignant tumor but they do not have the destructive tendency of a malignant cell. They are instead the various types of immature cells sent into the periphral circulation probably because of some improper activation of the formative tissue.

Many authors are of the opinion that the chronic forms belong in the class of neoplasms, but the microscopical picture does not bear out such a contention. Although these cells are large and irregular they show no invasive tendency and do not invade the surrounding structure. In figure 4 showing a section of kidney in a case of lymphatic leukemia, there can be seen marked lymphocytic infiltrations which push aside and separate the tubules and glomeruli but do not invade and destroy them as occurs in malignant tumor. These infiltrations are accumulations of cells which resemble the picture produced by infection rather than the one produced by an invading tumor. It seems quite plausible that the various forms of leukemia and the diseases listed under blood dyscrasies have their origin in an infectious process. It is further conceivable that some body constituent or modification of the normal defense reaction in the body interferes with the normal activation of the manufacture and destruction of cells of the formative tissue.

The following diagram is presented to summarize these observations:

HAEMATOPOESIS

Infectious Leukocytosis (normal producing capacity)   Erythrosis   Reticulo-endotheliosis

Agranulocytosis (hypo-producing capacity)   Aplastic Anemia   Leukemic   Aleukemic

Leukemia (hyper-producing capacity)   Reticulosis or Mononucleosis   Hodgkin's Lympho-granuloma

Lymphatic   Myelogenous
Acute, chronic or aleukemic
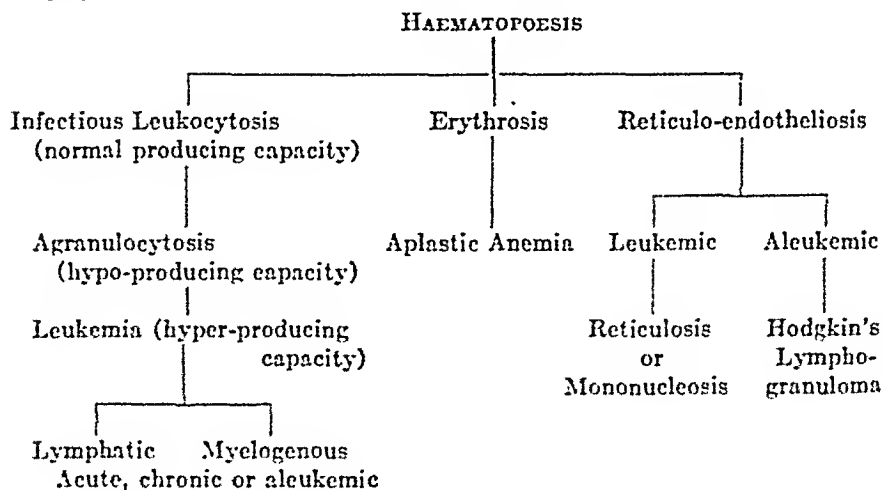
CONCLUSIONS

1. There is a constant physiological balance between the manufacture and destruction of blood cells; this process maintains an equilibrium which is compatible with health.

2. Introduction of a foreign substance, as for instance, infection, stimulates the formative tissue to greater production of blood cells; this may be called the normal producing capacity. Some abnormal body constituent may account for the lack of proper activation of these formative tissues, which results in some form of blood dyscrasia.

3. In the leukemias there is an hyperproducing capacity with over production of cells whereas in agranulocytosis there is an hypo-producing capacity with lack of cells. The histopathology of all leukemias indicates that there is no essential difference between the acute, chronic and aleukemic forms.

4. Many septic processes seem to produce the same clinical symptoms and histological changes that are seen in diseases of the hematopoetic system. Aplastic anemia, agranulocytosis and mononucleosis are usually associated with septic processes while Hodgkin's disease presents histological evidence of inflammation.

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GELATINOUS CARCINOMA OF THE BREAST

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Gelatinous carcinoma of the breast has recently been reviewed by Cheatle and Cutler,[2] who conclude that the origin of the gelatinous material is epithelial. That is to say, the gelatinous material is a product of degenerative changes within the tumor cells, or a product of their metabolism. The pathogenesis of this form of carcinoma of the breast has been the subject of some controversy. Cheatle and Cutler reviewed the literature, and their findings need not be repeated here. Suffice it to say that two opinions have been held. Some maintain that the gelatinous material is the result of stroma degeneration, and others maintain that the epithelial cells alone are responsible.

The opinion has been held, too, that these tumors are of a lower grade of malignancy than other carcinomas of the breast. Cheatle and Cutler maintain that they are as malignant as other carcinomas, and further point out that areas of gelatinous degeneration may be found in many forms of carcinoma of the breast. Recently, a case of gelatinous carcinoma of the breast was encountered in the laboratory, and because of its size and rather uniform histology, it was thought worthwhile to report it.

CASE REPORT

The tumor was obtained by operation from a woman thirty-nine years of age, who four years before had noticed a small nodule in the left breast. This nodule was quite hard, but not tender or painful. In the past year the tumor had grown very rapidly, so that at the time of operation the entire breast was involved in a hard, indurated tumor mass, the skin surface of which had ulcerated. Two months prior to the operation, a small nodule appeared in the right breast, and the left axilla was discovered to contain numerous hard glands. The tumor was now quite painful and obviously infected, at least superficially. In view of the nodule in the right breast and the axillary masses, radical operation was not performed, but only simple excision of the tumor in order to relieve the patient of pain and the complications of secondary infection.

The specimen (fig. 1) consisted of a breast occupied by a tumor measuring 10 by 8 by 6 cm. The skin was wrinkled and indurated, superficially ulcerated and slightly nodular. The cut surface revealed a diffuse growth of strikingly gelatinous appearance permeated by fine strands of stroma, giving an irregularly lobulated appearance. It was of pale gray color and resembled the cut surface of an indurated colloid goiter of considerable duration, except that it lacked the usual pink luster of a colloid goiter.

FIG. 1. GROSS SPECIMEN

Microscopic examination of a great many sections revealed a uniform histological picture. Coarse strands of acellular connective tissue made up the stroma. In places this stroma (fig. 2) was rather well preserved and took a deep eosin stain, whereas in other areas it stained poorly and seemed to have undergone degenerative change. The stroma was separated by masses of nonstaining material. At intervals, dilated ducts lined by flattened or slightly cuboidal and low columnar epithelium were present. Lying loosely in the gelatinous material
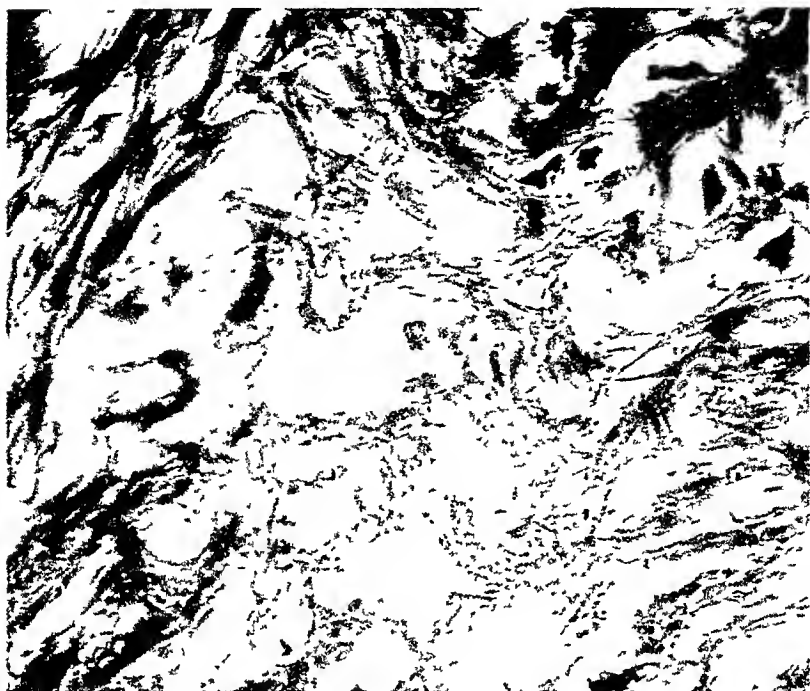
FIG. 2. PHOTOMICROGRAPH OF TUMOR
Note stroma

FIG. 3. PHOTOMICROGRAPH OF TUMOR
Note cytoplasmic vacuolization and nuclei crowded to one pole

were clusters of tumor cells.   In the main, these cells maintained the appearance of small ducts or alveoli, but in places were arranged in solid nests.   It was in these solid nests that the gelatinous material seemed to take origin.   The cytoplasm of the cells was vacuolated.   Frequently, the cells were massed together, forming a syncitial mass of tumor cells in which the cytoplasm had become vacuolated and the nuclei crowded together at one pole of the cell mass (fig. 3). The tumor cells were fairly uniform, with rather large oval and circular nuclei having a fine chromatin network and rather prominent nucleoli.   Amitotic and mitotic division were occasionally noted.   In some places the cells were so massed together as to have lost their outline, leaving only large numbers of nuclei imbedded in a mass of cytoplasm.   It was frequently in these areas that the gelatinous formation seemed to be most active, although it occurred in even smaller groups of cells with only two and three nuclei.   In some areas the cell cytoplasm had completely disappeared, leaving only shadowy remnants of the nuclei, which were in turn degenerating.   Occasionally, extrusion of the vacuole seemed to be taking place, the vacuole bulging from the membrane of the cell.   The sections from the deep portions of the tumor did not reveal any secondary inflammatory reaction, but the skin surface was the seat of a mild chronic inflammatory infiltration.   In some of the ducts there was proliferation and piling up of the epithelium into the lumen.   Here the cells were very large, the cytoplasm was vacuolated and closely resembled columnar cells of the colloid-secreting type. In some areas the syncitial masses of tumor cells were so filled with the gelatinous material that the cytoplasm was spread out in a thin shell, giving the appearance of minute cysts, with the remnants of the nuclei imbedded in the remnant of the cytoplasmic mass.   Sometimes the nuclei were lying free in the vacuoles.   In these areas there was lacking any evidence or else there was very scanty evidence of previous stroma.   The stroma seemed to have been displaced and condensed to form denser bands, as described previously.   A few tumor cells could be seen in what appeared to be lymphatic spaces, and occasional masses of atrophic tumor cells seemed to be imbedded in the denser stroma, giving an appearance suggestive of scirrhous carcinoma.   Although the tumor was not richly cellular, the cells seemed to be vigorous and there was little evidence of spontaneous degeneration or necrosis.

Ewing mentioned that such tumors frequently metastasize as adenocarcinoma.   Special staining of the tissue did not reveal any fat in the epithelium, and an extremely scanty amount in the stroma.   The gelatinous areas stained with mucicarmen, and mucicarmen stained faintly, but not uniformly, some of the intracellular globules.   It did not stain the intraductal secretion.

It would seem from this specimen that the conclusions of Cheatle and Cutler are supported.   It is my opinion that the gelatinous material is a product of cell metabolism and not necessarily one of cell degeneration.

The patient survived for at least eight months after the operation. Since that time I have been unable to ascertain her condition. The history of the tumor is slightly less than five years. It seems reasonable to suppose that had the patient been operated upon when the tumor was first noted, cure might have been expected, since the slow development and the late appearance of metastases indicate a relatively benign form of carcinoma. From the sections it would seem that this tumor is of duct origin. The histology does not, in our opinion, necessarily indicate a low degree of malignancy, but since the tumor was four years old, it is quite possible that its primary cell form was different from that noted in the specimen.

Kaufman[4] was rather inclined to attribute the origin of the gelatinous substance to both degenerative changes in the stroma and secretory or degenerative changes in the epithelium. Ewing,[3] in four cases personally examined, was unable to attribute the origin of the gelatinous material to the epithelium. P. d'Allaines, Funck-Brentano and Pavie[1] reported an instance of colloid or gelatinous carcinoma of the breast occurring one year following the removal of a benign adenoma from the same breast. There was no evidence of any mucoid or gelatinous change in either the stroma or the epithelium of the benign adenoma. In the carcinoma, the gelatinous change was extensive, and there was evidence of the gelatinous substance in the lumen of the alveoli and in some of the terminal ducts. The authors were inclined to consider that the origin of the gelatinous material was in the stroma, but did not deny the possibility of primary epithelial change. Lazzarini[5] reported three cases of gelatinous carcinoma, and attributed the origin of the gelatinous material to the stroma. He was unable to find any evidence of primary change in the epithelium, and considered that the nutrition of the carcinoma cells was considerably interfered with by the degenerative changes in the stroma.

In the case reported here it seems important to note the goblet type of epithelium in some of the ducts, both in those which had undergone hyperplasia and in those which showed very little hyperplastic change.

Numerous sections taken from a considerable number of surgically removed breasts not involved by carcinoma and a

fewer number of samples of breast tissue removed at autopsy failed to reveal, either in the main ducts or in the deeper portions of the breasts, any goblet epithelium. The possibility that such epithelial cells may indicate an origin from sweat gland epithelium was considered, but we have been unable to detect any such epithelial changes in sections of sweat glands examined for a variety of lesions. It seems, therefore, that one is reduced to two possibilities to account for these goblet cells and the gelatinous secretion. One is degenerative change, for which there is considerable evidence, and the other is a functional and structural metaplasia. The former is the easier to accept, especially in view of the fact that areas of gelatinous change are frequently encountered in many types of carcinoma. However, the involvement of the entire tumor lends some support to the theory that this substance may not be the result of degenerative changes, but actually the result of a secretory function of the epithelium which has undergone metaplasia, or rather differentiation from the columnar epithelium to an actively secreting columnar epithelium. It is to be regretted that some of the metastases were not removed and examined, since it would be interesting to know if this tumor reproduced itself, and whether the metastases were of the adenocarcinoma type.

SUMMARY

An example of colloid or gelatinous carcinoma of the breast is described. The gelatinous material is interpreted as a product of the tumor cells.

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THE COLLOIDAL BENZOIN TEST OF CEREBROSPINAL FLUID

Its Clinical Value

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The colloidal benzoin test has been in use in the laboratory of the Los Angeles General Hospital for more than four years. This study is based on data collected from the first 2000 of these tests which were applied to routine spinal fluids from some 1800 patients. The study will be chiefly limited to the constancy and specificity of this reaction in syphilis of the central nervous system, poliomyelitis, tuberculous meningitis, epidemic encephalitis, and purulent meningitis. One may find complete references on the colloidal benzoin test in the excellent bibliography by Kermack and Voge.[8]

Keidel and Moore[7] in reporting the results from 311 cases considered the gum mastic test more delicate than the colloidal gold. Warnock[17] after studying eighty-seven cases was unfavorable toward benzoin and considered the gold test more reliable. Riddell and Stewart[15] from 100 tests were of the opinion that the benzoin was more delicate than the gold test. Wright and Kermack[19] found substantial agreement between the gold and benzoin tests and concluded that the latter was simpler and in many ways more satisfactory for clinical use. After studying the results from more than 1700 cases, Wassermann[18] considered the mastic equal to or more delicate than the gold test. From a series of 400 tests, Cockrill[1] was of the opinion that the mastic and benzoin tests were each of equal value to the gold test and that the benzoin test was simpler. Osborne,[10] after a comparative study of 1000 gold and benzoin tests, and of an additional 1000 benzoin tests alone, concluded that benzoin is superior to gold in ease of performance, and is more uniform, reliable, and

informative. Reyner[14] concluded that while the gold test is more informative than the mastic, although not more delicate, the benzoin test is the most delicate of the three, and more informative than the mastic test. Kermack and Voge[8] were still of the opinion that the benzoin test was easier to prepare, was simpler, and offered less likelihood of error and more likelihood of uniformity of results in routine laboratory work, than did the colloidal gold test.

The evidence therefore seems to favor the benzoin test as the simplest to prepare and read, as being as sensitive as and more informative than the mastic test and more sensitive than and as informative as the gold test.

TECHNIC

The original technic of Guillain and Léchelle[5] was used, as follows:

Glassware

Bottles, tubes (1 cm. by 6 cm.) 1 cc. and 10 cc. pipettes, all of nonsoluble glass. To be washed before using in 2 per cent aqueous hydrochloric acid and then twice in distilled water.

Solutions

Two solutions are needed: (1) a saline solution containing 0.1 gram of chemically pure sodium chlorid to 1000 cc. of twice distilled water; (2) a homogenous suspension of the resin of benzoin in distilled water, prepared as follows:

Dissolve 1 gram of the natural resin of benzoin (Sumatra) in 10 cc. absolute alcohol for forty-eight hours. The limpid liquid obtained is poured off and preserved as a stock solution. When a reaction is to be performed, 0.3 cc. of this alcoholic solution is added slowly to 20 cc. double distilled water heated to 35°C. in such a manner as to obtain a very homogeneous suspension. This must be freshly prepared and must not be kept over a couple of days. The water used must have been recently distilled.

Actual technic of reaction

Line up fifteen tubes with one extra for control.

To tube 1, add 0.25 cc. of the saline solution
To tube 2, add 0.50 cc. of the saline solution
To tube 3, add 1.50 cc. of the saline solution
To tubes 4 to 16 inclusive, 1 cc. of saline solution

Then pipet, shaking the mixture carefully after adding, the following amounts of spinal fluid:

To tube 1, 0.75 cc. spinal fluid
To tube 2, 0.50 cc. spinal fluid
To tube 3, 0.50 cc. spinal fluid

Then take 1 cc. of the contents of tube 3 and, having aspirated a few times to mix, transfer to tube 4. Mix the contents of tube 4 and transfer 1 cc. to tube 5. Continue this until tube 15 is reached, and reject 1 cc. from this tube, leaving tube 16 as a control.

Into each tube put 1 cc. of the benzoin suspension. The contents of all the tubes will be milky in appearance.

Place in ice-box and read in from six to twenty-four hours.

Interpretation

In the greater part of this series four numerals were used in recording the tests, namely, 0, 1, 2, and 3. A tube showing no precipitation was recorded as 0, a slight precipitation was called 1, almost complete precipitation but with cloudy supernatent fluid was designated by 2, while complete precipitation with clear supernatent fluid was read as 3. Three numerals only, 0, 1, and 2, were used in recording the earlier tests of the series; the 2 included both the later 2 and 3. In order to make the results uniform all 3's have been reduced to 2's throughout the series.

A normal reaction is usually indicated by a complete series of zeros; but as described by Guillain and Léchelle[5] there may normally be some precipitation in one or two tubes of numbers 5 to 8 inclusive. We have considered as normal precipitation less than grade 2 occurring in not more than three tubes in a zone.

Sources of error

Guillain et al.[3] repeated the colloidal benzoin test on cerebrospinal fluids kept at laboratory temperature for many days with no appreciable alteration in results. We have repeated the benzoin test on spinal fluids kept in the ice-box for one week with no appreciable difference in the curve providing the fluids remained sterile. It is probably best, however, to use spinal fluids not more than a few hours old to avoid the possibility of changes which may presumably alter the curve.

Schaffer[16] believed that precipitation of benzoin was due to variation in the hydrogen ion concentration of spinal fluid, or of the colloidal suspension. Papayanno[11] demonstrated the globulin factor in benzoin precipitation with spinal fluid.

Blood plasma in the spinal fluid will produce a curve in a normal fluid and alter a primary curve. The readings a, b, and c shown in table 1 were obtained by adding small amounts of the patient's blood plasma to the spinal fluid. The

plasma-free fluid used as a control gave a normal reaction with the benzoin. Bloody spinal taps probably constitute one of the greatest sources of error.

Because of its simplicity, technical errors are less likely to occur with the colloidal benzoin than with other similar "colloidal" tests and reading is dependent upon a precipitation rather than a change in color.

Zones

The fifteen tubes of the colloidal benzoin have been divided into two zones. The first zone comprises tubes 1 to 6, and the second zone includes tubes 7 to 15. The second zone is also called the tabetic and meningitic zone. The first zone has been called the paretic zone. Osborne found that a positive first zone reading in syphilis of the central nervous system usually meant general paresis, whereas in other forms of neurosyphilis the precipitation was confined to the second zone; also a positive first zone reading was considered to be an index of the degree of active involvement of the parenchyma of the brain, whereas purely vascular, meningeal, or spinal cord lesions produced precipitation in the second zone. Jaffrey[6] similarly concluded that there was this distinctive difference in zones in tabes and paresis, and that apparently the reaction was of value in finding where these two types of syphilis invade each other's territory.

A total of 2009 benzoin tests were run on fluids from 1800 cases, including 152 cases of syphilis of the central nervous system, 116 of tuberculous meningitis, 135 of poliomyelitis, nine of encephalitis, 316 of purulent meningitis, 616 miscellaneous diseases, and 456 cases in which the diagnosis was uncertain or complications were such as to make the case unsuitable for study.

SYPHILIS OF THE CENTRAL NERVOUS SYSTEM

Of the 152 cases of syphilis of the central nervous system diagnosed clinically, all gave positive colloidal benzoin readings. The spinal fluid Wassermann was positive in 142 and negative in ten cases. The spinal fluid Wassermann had been positive in seven of the ten negative cases within the past three years and had become negative evidently as a result of treatment. The blood Wassermann was positive in the remaining three cases with negative spinal fluid Wassermann and no past records of spinal fluid examination were available.

In order to study the benzoin curves, all cases of this type of syphilis were divided into three groups according to the clinical diagnosis, namely general paresis, tabes dorsalis, and meningovascular syphilis.

General paresis. The colloidal benzoin test was applied to spinal fluids from seventy-six cases of paresis; thirty-eight cases were from our routine series and thirty-eight additional cases were obtained from the Norwalk State Hospital through the courtesy of Dr. Melvin J. Rowe of that staff. All cases of paresis were divided into two groups, those having received treatment for less than one year, and those having been treated for one year or more.

There were forty-seven cases in the first group and most of these were practically untreated. Forty-six or 97.9 per cent gave

TABLE 1

REPRESENTATIVE BENZOIN READINGS

Normal spinal fluid		000000000000000
Normal fluids—blood contamination	(a)	000001222222222
	(b)	222222000002222
	(c)	012222222222222

Average group curves

General paresis (little or no treatment)	122222222221110
Tabes dorsalis	000001222221100
Meningo-vascular syphilis	000001222221000
General paresis (treated on average 4 years)	000001222110000
Tuberculous meningitis	000001222222100
Poliomyelitis	000000222110000
Epidemic encephalitis	000000000000000
Purulent meningitis	111111111211111
Three types of curve in purulent meningitis (1)	000000222210000
(2)	111222000222221
(3)	111112222222222

a definite first zone reading, that is, precipitation occurred in three or more of the first six tubes, and in addition the curve extended to a varying degree into the second zone. All first zone readings were quite positive and for the most part the individual curve corresponded quite closely to the average curve for the group (table 1). Although all the readings extended into the second zone, the precipitation was as heavy in the paretic zone as in the succeeding zone and the curve was continuous without a drop in the middle. One case is of especial interest in that

the spinal fluid Wassermann had become negative after one year of treatment while the benzoin gave a typical paretic curve.

The twenty-nine cases comprising the second group of paretic patients had on an average received treatment over a period of four years. A positive second zone reading was obtained in each case but only six cases gave paretic zone readings. The average curve for the group is shown in table 1. The spinal fluid Wassermann was positive in all but five cases in which it had become negative during the period of treatment.

Tabes dorsalis. There were forty-three cases of tabes and each gave a positive second zone reaction in three or more tubes. Precipitation started in either tube 6 or 7 and the individual curve corresponded quite closely to the average for the group (table 1). In only two cases did the precipitation occur in three or more tubes in the paretic zone. Clinically, recent mental changes had been noted in each of these two patients. The spinal fluid Wassermann was positive in all but one case of the group, and in that one it had become negative during two years of treatment.

Meningovascular syphilis. This group included thirty-three cases each of which gave precipitation in three or more tubes in the second zone and corresponded in general to the readings in the tabetic group.

The paretic zone. In the entire series, including syphilis of the central nervous system there was but a single case, one of cerebellar tumor, in which precipitation was confined to the first zone. However, aside from these cases of syphilis and those of purulent meningitis, there were seventeen cases in the entire series having benzoin curves simulating curves in general paresis (table 2). These included one case each of cerebellar tumor, multiple sclerosis, tetanus, torula meningitis, four cases of coccidioides meningitis; and nine cases of tuberculous meningitis. Guillain et al.[4] in reporting the results of the colloidal benzoin on spinal fluids from twenty-eight patients with cerebral tumor, found no curves of the paretic type; the benzoin was negative in five cases, precipitation was present in the second zone only in seventeen cases, and was present in both the first and second zones with negative tubes between the zones in six cases. Neither is the paretic curve

the rule in tetanus since in our series there were twenty cases, the curve in only one of which somewhat simulated paresis. A case of multiple sclerosis was one out of three. The nine cases of tuberculous meningitis were from a group of 116 cases. The four cases of meningitis due to coccidioides and the one case of torula meningitis represented all of the cases of these diseases.

TABLE 2

Cases, not Syphilis, with Paretic Type Curve

NUMBER	CURVE	DIAGNOSIS
1	210000000000	Cerebellar tumor
2	902002222200000	Multiple sclerosis
3	111211222211111	Tetanus
4	000222211222210	Tuberculous meningitis
5	112212222222222	Tuberculous meningitis
6	222202222222222	Tuberculous meningitis
7	122222222222222	Tuberculous meningitis
8	022222222222000	Tuberculous meningitis
9	222222202222222	Tuberculous meningitis
10	000122222222100	Tuberculous meningitis
11	012222222222222	Tuberculous meningitis
12	222202222222222	Tuberculous meningitis
13	122222222222210	Coccidioides meningitis
14	000222221222222	Coccidioides meningitis
15	222202222211000	Coccidioides meningitis
16	012222222222222	Coccidioides meningitis
17	000002222222221 0122222222222222	Torula meningitis

TUBERCULOUS MENINGITIS, POLIOMYELITIS, AND EPIDEMIC ENCEPHALITIS

These diseases will be considered in one group because of their clinical relationship, and because in certain instances there is difficulty in the differential diagnosis.

Tuberculous meningitis. Benzoin tests were run on specimens of spinal fluid from 116 patients. A positive reading was obtained in 97.4 per cent. Three cases in which the spinal fluid findings were otherwise consistent with the clinical diagnosis gave a normal benzoin reaction, and although the tubercle bacillus was not found in the spinal fluid, these cases were clinically

diagnosed as tuberculous meningitis and went on to a fatal termination. The average curve for the series is shown in table 1 and occupies the second zone. Readings in eleven cases gave curves simulating that found in cases of general paresis in that heavy precipitation started in the first zone, involved three or more tubes, and continued uninterrupted into the second zone. Two of these cases had a positive spinal fluid Wassermann. The remaining nine are charted in table 2. They were all terminal cases at the time the spinal fluid was taken.

Poliomyelitis. This group included 135 cases of poliomyelitis in which the colloidal benzoin test was applied to the spinal fluid within the first two weeks of the disease. Ninety-four and seven-tenths per cent gave a positive reaction in the second zone. There were no first zone reactions. Occasionally a negative reading was obtained on the first or second day of the disease, but when the test was repeated the following day the result was a typical curve. The individual curves were quite uniform, and in the main corresponded very well to the average curve for the group (table 1).

In seven cases the benzoin test was normal; the spinal fluid was taken sometime between the first and seventh day of the disease, and examination of the spinal fluid was otherwise consistent with the clinical diagnosis. Paralysis was present in five of the seven cases.

These results are quite similar to those of Regan[13] who obtained a positive reaction in twenty-two of twenty-three cases of poliomyelitis and the zone of reaction was in tubes 6 to 9, often extended to 10, and occasionally to 11 and 12. He found negative tests were of increasing frequency after the fourth week of the disease, and spinal fluids in the sixth week were normal.

Encephalitis. There were nine cases, four of epidemic encephalitis, two of encephalitis following influenza, two of encephalitis complicating measles, and one case of the Marie Strümpell type. The spinal fluid from each of these patients gave a normal benzoin reaction except that from a patient having complicated measles by encephalitis, in which a positive second zone reaction was obtained.

Guillian and Léchelle reported three cases of epidemic encephalitis and a fourth post-encephalitic case in each of which the colloidal benzoin reaction was normal. Six cases of epidemic encephalitis were reported by Duhot and Crampton,⁸ and ten cases by Rabeau,¹⁵ in all of which the colloidal benzoin was negative. Ménard⁹ found the benzoin constantly negative in epidemic encephalitis and positive in tuberculous meningitis.

Whereas the average curve of the group in poliomyelitis differs somewhat from the average curve of the group in tuberculous meningitis, the individual curve in the one condition may simulate the average curve of the other in many instances and therefore it is questionable if the benzoin test is of value in differentiating between these two conditions.   However, there is evidence that the colloidal benzoin test may be of considerable value in the differentiation of epidemic encephalitis from poliomyelitis and tuberculous meningitis.

PURULENT MENINGITIS

The colloidal benzoin test was applied to the specimens of spinal fluid from 316 patients having purulent meningitis.   There were 221 cases of the epidemic type and ninety-five miscellaneous cases of which the pneumococcus was the most frequent cause. The test was positive in 99 per cent of cases.   The precipitation was not confined to one zone although heavier in the second zone. The individual curve did not correspond very closely to the average curve for the group which involved all fifteen tubes (table 1). Early in the disease however, the reading was usually confined to the second zone.   Later precipitation extended to the end of the second zone, and forward into the first zone.   Not infrequently a double curve resulted with precipitation in practically all tubes except three or four in the middle.   The various types of purulent meningitis were indistinguishable as far as the colloidal benzoin reaction was concerned.

MISCELLANEOUS GROUP

This group comprised 616 cases, 150 of which were diseases of the central nervous system.   In general, fluid from patients with

organic disease of the central nervous system gave positive readings confined to the second zone with the exception of those cases discussed in relation with the paretic zone. The group included cases of multiple sclerosis, subacute combined sclerosis, bulbar palsy, cerebral hemorrhage and thrombosis, epilepsy, rabies, botulism, tetanus, psychoses, et cetera.

In the great majority of miscellaneous diseases other than those of the central nervous system there was a normal benzoin reaction, as might be expected. However, it was found that certain types of disease were apt to have a second zone curve, namely: infectious diseases such as lobar pneumonia accompanied by meningismus, diseases such as diabetes mellitus and nephritis, accompanied by acidosis or nitrogen retention, and acute inflammatory processes adjacent to the dura mater such as sphenoid sinusitis, and mastoiditis in which the spinal fluid shows no bacteria, although there may be a slight increase in lymphocytes and globulin.

SUMMARY

(1) The evidence in the literature indicates that of the colloidal tests used in examination of the spinal fluid the benzoin test is the simplest to prepare and read, is as sensitive as and more informative than the gum mastic test, and is more sensitive than and as informative as the colloidal gold test.

(2) A study of the data obtained from some 2000 colloidal benzoin tests on cerebrospinal fluids from 1800 patients signifies that: (a) the benzoin test is not a specific test in the same sense as the Wassermann reaction, (b) it is of value in differentiating active general paresis from other forms of neurosyphilis, (c) it is probably of value in differentiating epidemic encephalitis from poliomyelitis and tuberculous meningitis, (d) a high percentage of positive readings is obtained in disease of the central nervous system, but a positive second zone reading is occasionally obtained in certain conditions not associated with organic disease of the central nervous system.

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EDITORIAL

Death Certificate Diagnoses

The importance of accurate mortality statistics to the health of a nation is fully appreciated by the educated layman as well as the physician. That the progress of medicine is dependent in a not insignificant measure on the accuracy of such statistics is a fact not so fully appreciated even by physicians, upon whom the responsibility for the accuracy of the figures depends.

Clinical diagnoses are seldom correct in their entirety even when made by physicians whose ability is unquestioned. When there is added to the excusable error of judgment a certain amount of carelessness and lack of knowledge of the principles of classification for mortality statistics the error becomes enormous. The result is that instead of mortality tables being helpful they may do harm because they are misleading. So great a factor in the public welfare and the progress of medicine deserves more than ordinary effort toward accuracy on the part of those who are responsible for the diagnoses written on the death certificates since these are the source from which mortality tables are derived.

Every pathologist of experience has witnessed the uncertainty, even confusion of the clinician in deciding upon the cause of death to be written on the death certificate. Too often the pathologist is content to write his anatomical diagnosis and watch in silence the efforts of his colleague to translate or qualify this anatomical diagnosis so that it will be acceptable to the registrar of his state.

For evidence that the difficulties of the physician are real the figures of Wood may be cited. He states that in Pennsylvania one in every forty death certificates is so incomplete as to require correspondence with the physician and that every year 4500 physicians are asked to correct their diagnoses or supply deficiencies. The chief reason for these difficulties of the physician

lies in the difference of his point of view from that of the public health official.

The physician is trained to think of disease as abnormality of function and structure and makes his diagnosis on this basis whereas a satisfactory diagnosis from point of view of the public health official must take into consideration the factors responsible for bringing about these abnormalities of function and structure.

It is for this reason that the diagnosis of bronchopneumonia not qualified by the adjective "primary" is so often returned for additional information. The registrar knows that bronchopneumonia is more often a terminal complication of some other condition and it is the primary disease that he wants. If bronchopneumonia follows an acute infectious disease such as measles or whooping cough he is more interested in the possibility of forestalling an epidemic than in the anatomical and functional cause of death—consolidation of the lungs by an exudate caused by a toxin producing streptococcus. Similarly a diagnosis of fracture of the skull with laceration of the brain will satisfy the pathologist and keeper of hospital records but the keeper of mortality records for the state must know the cause of the fracture, not only the manner in which it was brought about but whether it was accident, suicide, or homicide. Again the idea of prevention is uppermost in his mind.

Pathologists can perform a real service if they will study the principles underlying the classification for general mortality statistics and familiarize themselves with the International List of the Causes of Death as adopted by the greater part of the civilized world including the Bureaus of Vital Statistics in the Registration Area of the United States. By doing this they will be in a position to assist the physician in what is at times a difficult task, increase the accuracy of important figures, and incidentally make additional friends.

<div align="right">W. S. Thomas.</div>

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The following will act as a program committee for the Twelfth Annual Convention of the A. S. C. P.: A. S. Giordano, Chairman, A. H. Sanford and F. W. Hartman. The local committee at Milwaukee will consist of Norbert Enzer, Chairman, E. L. Tharinger, E. F. Barta and Marcos Fernan-Nunez.

During the absence of Dr. H. C. Schmeisser, Dr. M. Pinson Neal, professor of Pathology at the University of Missouri School of Medicine, has served as visiting professor in the Department of Pathology at the University of Tennessee College of Medicine and as acting director of the laboratories of the Memphis General Hospital.

Dr. John A. Kolmer has been appointed professor of Medicine at Temple University.

BOOK REVIEWS

The Chemistry of Tuberculosis. 2nd Edition. By H. Gideon
Wells and Esmond R. Long. Pp. xiv + 481, 1932, Balti-
more, The Williams & Wilkins Company, $7.00.

This well known work which is essentially a review of literature,
has been thoroughly rewritten to include the newer views con-
cerning the chemistry of tuberculosis. The book has been en-
larged by one new chapter and thirty-two pages of text. But this
does not indicate the extent of the changes, for one notes that
whereas the original text contained about 1100 references to liter-
ature, the present edition contains nearly 1700 which indicates
that about seventy-five contributions have appeared every year
since the first edition. In the chapter on the growth and metab-
olism of *M. tuberculosis*, more than 100 new references appear.
The book represents an excellent summary of all that is known of
the chemistry of this disease and its causative organism. The
chapters are not only summarized, but the contents of each are
critically reviewed in a few brief paragraphs.

Human Cancer. By Arthur P. Stout. Pp. viii + 1007, 1932,
Philadelphia, Lea & Febiger, $10.00.

This monograph approaches the subject of cancer from a differ-
ent aspect than most texts on the subject. The author has pre-
sented his material by regions of the body, discussing the malig-
nant growths of each region in relation to etiological factors,
precancerous lesions, growth, spread, symptoms, diagnosis, prog-
nosis and principles of treatment. He has accepted the factor of
chronic irritation as a working hypothesis for the cause of cancer
which he discusses at length. Most of the highly speculative
phases of the subject are omitted as well as academic experimental
cancer research. A well chosen bibliography at the end of each
chapter, records the literature by years during the past quar-
ter century or more, the author, with rare honesty admitting that

it is not complete and that he has not read it all. The illustrations, numbering more than 300, are original and excellent. An appendix by G. F. Laidlaw gives the technic for silver staining.

The book exhibits a great amount of labor and intelligence on the part of the author and makes a practical, yet extensive, modern treatise on cancer, useful not only to the surgeon and pathologist, but to the internist and radiologist.

Quantitative Clinical Chemistry. Volume II, Methods. By JOHN P. PETERS AND DONALD D. VAN SLYKE. Pp. xx + 957, 1932, Baltimore, The Williams & Wilkins Company, $10.00.

This is a fitting companion to the previously published volume on "Interpretations." In it one finds the exact steps, given in detail, for carrying out the tests, the results of which were discussed in the first volume of this monumental work.

The book contains a clear exposition of the use of the equipment needed to perform quantitative clinical chemical tests and includes the answers to hundreds of technical questions that arise in the minds of those performing biochemical procedures. As would be expected the authors devote a generous share of the work to gasometric methods on which they are leading authorities. But for the most part at least one gravimetric, titrimetric and colorimetric method is given for the determination of each substance. The style of presentation is excellent, direct and clear; each test is described by giving the apparatus required, the reagents, the procedures and the exact method of calculation to obtain the result. Illustrations, tables, nomograms and references are plentiful.

It is unfortunate that so excellent a text should have so poor an index, for many items would never be suspected of being present were one to consult the index alone. The same condition also prevails with reference to the author index. There are some omissions in the text also as for example tests for enzymes, carotin, certain heavy metals such as lead and arsenic, nitrates and nitrites, bile salts and acids, and there is no discussion of sugar tolerance tests and the Congo red method for blood volume determinations. There is an evident error in the description of the diazo reaction where the color is described as being blue. But these omissions seem trivial when the book is considered as a whole.

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